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A MATHEMATICAL THEORY OF CHROMOSOMAL REARRANGEMENTS*

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(With Two Text-figures)

When a nucleus is exposed to X-rays, gene mutations and minute inversions and deficiencies are produced roughly in proportion to the dose. Large rearrangements, including inversions and translocations, occur with a frequency increasing more rapidly than the first power of the dose but (in *Drosophila*) less rapidly than its square (Muller, 1940, 1941). In the case of *Drosophila* spermatozoa a given dose in roentgens gives the same result regardless of its intensity or of intervals during its application. In other cases this is not so, since fragments appear to join during irradiation. It is generally agreed that the number of breaks primarily produced in the chromosomes of a nucleus is proportional to the dose (e.g. Sax, 1940; Carlson, 1941), and that large rearrangements are due to unions of two or more breaks. In *Drosophila*, at any rate, broken ends rarely unite with normal ends, and terminal deficiencies are also rare, though they seem to occur more frequently in *Zea* (Stadler, 1941).

The theory developed in this paper rests on the following assumptions (cp. Pontecorvo, 1942; Lea & Catcheside, 1945). We suppose that after a dose D of radiation, an average of αD breaks per nucleus are produced, made up of an average number αpD breaks which undergo sister-union and behave as dominant lethals in the manner envisaged by Pontecorvo and Muller, and an average number αqD breaks which do not undergo sister-union. $p+q=1$, and α, p and q are assumed to be constants which are independent of the dose (though possibly dependent on the state of the nucleus at the time of irradiation); and union among the breakage ends which do not undergo sister-union is presumed to be random. The assumption of random union of breakage ends will not be a valid approximation in all cases. It is fairly certain, for example, that in *Tradescantia* microspores union of breakage ends is far from random, but is mainly limited to breakage ends separated at the time of breakage by a distance less than one-tenth of the nuclear diameter (Lea & Catcheside, 1942). There is reason for believing, however, that union may be more nearly random in some other cases, particularly after irradiation of *Drosophila* sperm. A preference for restitutional union as opposed to unions resulting in chromosome structural changes is not incompatible with the present calculations, providing it can be assumed that the mean number of such preferentially restituting breaks is proportional to the dose. In this event αqD is interpreted to be the number of randomly uniting breaks remaining after subtraction of the preferentially restituting class of break.

* This paper was first drafted (by J. B. S. H.) in 1942, but was not published as he was unable to evaluate the factor q. Subsequently Lea & Catcheside (1945) working on the same problem obtained results which were, in part, identical.

Their calculations, however, involved the assumption that every break is in a different chromosome arm, which is strictly only a valid approximation when the number of arms is large compared with the number of breaks in the nucleus. It has been thought worth while, therefore, to complete the present paper, since the method it describes is not subject to this limitation, but can be applied to nuclei with any given number of arms, as well as to nuclei with many arms.

Rearrangements which are *eucentric* (or *symmetrical*), that is to say in which all the chromatin is attached to a centromere and in which therefore none is attached to two centromeres, will be viable. Arrangements which are *dyscentric* (or *asymmetrical*) will make a further contribution to the dominant lethals.

Rearrangement after r breaks

In this section we limit attention to nuclei in which no breaks undergo sister-union. First consider the total number of possible arrangements after r breaks. There are $2r$ breakage ends to unite in pairs. The first can unite with any of $2r-1$ partners, the third with any of $2r-3$, and so on. Thus the number of possible arrangements is

$$1.3.5.....(2r-3)\,(2r-1)=\frac{(2r)!}{2^r r!}. \tag{1}$$

This is so regardless of whether the breaks occur in the same or in different chromosomes. We have assumed that all arrangements are equally probable.

But only some are eucentric, including of course the original arrangement. The number of eucentric arrangements varies with the structure of the nucleus. We shall show that it is minimal when all the breakage ends are in different chromosomes or different arms of metacentric or V-shaped chromosomes, and maximal when all are in one (or two) telocentric or rod-shaped chromosomes, or in one (or two) arms of V-shaped chromosomes.

If the r breaks are all in different arms, there are r acentric breakage ends to be united with r breakage ends connected with the centromeres. Clearly they can be attached in $r!$ ways. Thus, if the number of chromosomes is large compared with r, a condition often fulfilled in practice, the frequency of eucentric arrangements is

$$\frac{1.2.3.....(r-1)\,r}{1.3.5.....(2r-3)\,(2r-1)}=\frac{2^r(r!)^2}{(2r)!}.$$

Since one of the $r!$ eucentric arrangements restitutes the original nucleus, the frequency of rearrangements giving eucentric nuclei with structural change is $\frac{2^r r!\,(r!-1)}{(2r)!}$.

If the r breaks all occur in one arm, there are $(r-1)$ fragments to be filled in between the centromere and the free end. They can be filled in in $(r-1)!$ orders. And each can be in the original direction, or inverted. Thus the total number of arrangements is $2^{r-1}(r-1)!$. This is equal to $r!$ if $r=1$ or 2, and exceeds it if $r>2$.

Now consider r breaks distributed over k arms. k terminal fragments are separated from the centromeres, and $r-k$ interstitial fragments are separated from centromeres and ends. If the centromeres and segments containing them are regarded as fixed we can arrange the k chromosome ends in relation to them in $k!$ ways, leaving k gaps. Into these k gaps, or some of them, $r-k$ fragments each with two breakage ends are to be fitted. The first can be put into any of the k gaps leaving $k+1$ gaps, the second into any of the $k+1$ gaps leaving $k+2$ gaps, and so on. Further, each of these $r-k$ segments can be placed in either of two directions. Thus the total number of arrangements is

$$k!\,k(k+1)\,(k+2)...(r-1)\,2^{r-k}=2^{r-k}(r-1)!\,k. \tag{2}$$

This expression has been given by Fano (1942). It is equal to $2^{r-1}(r-1)!$ if $k=1$ or 2, otherwise it lies between $2^{r-1}(r-1)!$ and $r!$. To sum up; if two breaks have occurred, the probability of a eucentric arrangement is $\frac{2}{3}$. If r breaks have occurred, the probability is

$\frac{2^{2r-1}\,r!\,(r-1)!}{(2r)!}$ for a nucleus containing one or two arms, and approximates to $\frac{2^r(r!)^2}{(2r)!}$ for one containing a large number of arms. For nuclei with an intermediate number of arms, the probability is intermediate.

We have now to calculate this intermediate probability, and also the frequency of inversions in a given chromosome. The former can be done with ease for the case where all the arms are equal. Consider the chromosome set consisting of three arms which are equal in the sense that the probability of a break in each is equal. If there are three breaks, the probability that they will all be in different arms ($k=3$) is $\frac{2}{9}$. In this case, the number of eucentric arrangements is $3!$ or 6. The probability that all the breaks are in one or two arms is $\frac{7}{9}$. In this case the number of eucentric arrangements is $2^2 2!$ or 8. Thus the mean number is $6\times\frac{2}{9}+8\times\frac{7}{9}=7\frac{5}{9}$, and since the total number of arrangements is 15, the frequency of eucentric arrangements is $\frac{68}{135}$. The mean number of eucentric rearrangements, i.e. of arrangements differing from the original, is $6\frac{5}{9}$, and their frequency is $\frac{59}{135}$.

In general, if $p(a, r, k)$ is the probability that all of r breaks will occur in k out of a total of a equal arms, $p(a, r, k)$ is a^{-r} times the number of terms in the expansion of

$$(x_1+x_2+\ldots+x_a)^r$$

which contain just k different x's. The expansion can be made by the multinomial theorem; alternatively tables are available facilitating the writing down of $p\,(a, r, k)$ for any set of values of a, r and k (Fisher & Yates, 1938, Table XXII, Initial differences of powers of natural numbers). For example, if there are five equal arms and six breaks, $a=5$, $r=6$, and we find $p\,(5,6,1)=\frac{1}{3125}$; $p\,(5,6,2)=\frac{124}{3125}$; $p\,(5,6,3)=\frac{216}{625}$; $p\,(5,6,4)=\frac{312}{625}$; $p\,(5,6,5)=\frac{72}{625}$.

The mean number of eucentric arrangements is

$$E_r=(r-1)!\sum_{k=1}^{a} p\,(a, r, k)\,2^{r-k}k.$$

Thus for five equal arms and six breaks the mean number of eucentric arrangements is

$$\frac{5!}{625}\,(25.2^4.2+216.2^3.3+312.2^2.4+72.2.5)=2245{\cdot}632.$$

The mean number of eucentric rearrangements (i.e. arrangements excluding the original one) is one less, or 2244·632.

In general, if there are r breaks in a chromosome set containing a equal arms, we proceed as follows. Of the a^r terms in the expansion of $(x_1+x_2+\ldots+x_{a-1}+x_a)^r$, a contain just one letter. Thus $p\,(a, r, 1)=a^{-r}.a$. The number of terms containing x_1 or x_2 or both, but none of $x_3, x_4, \ldots, x_a$ is obtained by putting $x_1=x_2=1$, $x_3=x_4=\ldots=x_a=0$, and is therefore 2^r. Subtracting the two terms (x_1^r and x_2^r) which contain x_1 or x_2 separately, we find that there are 2^r-2 terms which contain both x_1 and x_2 but no other letter. Hence there are $\binom{a}{2}(2^r-2)$ terms which contain just two letters, $\binom{a}{2}=\frac{1}{2}a\,(a-1)$ being the number of ways of selecting two letters from the total of a letters. Thus $p\,(a, r, 2)=a^{-r}\binom{a}{2}(2^r-2)$.

Again, there are 3^r terms which contain any or all of x_1, x_2, x_3, but none of $x_4, x_5, \ldots, x_a$. Subtracting 3 for the terms in x_1, x_2 or x_3 alone, and $3\,(2^r-2)$ for the terms in x_1 and x_2, x_2 and x_3, x_3 and x_1, we find that there are $3^r-3.2^r+3$ terms which contain all of x_1, x_2, x_3,

and none of $x_4, x_5, \ldots, x_a$. Thus $p(a, r, 3) = a^{-r}\binom{a}{3}(3^r - 3.2^r + 3)$, $\binom{a}{3} = \frac{1}{6}a(a-1)(a-2)$ being the number of ways of selecting 3 letters from the total of a. Thus

$$E_r = \frac{(r-1)!}{a^r}\left\{\binom{a}{1}2^{r-1} + \binom{a}{2}(2^r-2)\,2^{r-2}2 + \binom{a}{3}(3^r - 3.2^r + 3)\,2^{r-3}3 + \binom{a}{4}(4^r - 4.3^r + 6.2^r - 4)\,2^{r-4}4 + \ldots\right\} \quad (3)$$

where the summation extends over a terms (terms after the rth vanish if $r < a$). In this way we find:

$$\left.\begin{array}{ll}
\text{For 1 or 2 arms:} & E_r^{\mathrm{I}} = E_r^{\mathrm{II}} = 2^{r-1}(r-1)!. \\
\text{For 3 equal arms:} & E_r^{\mathrm{III}} = 3.2^{r-3}(r-1)!\left\{1 + \dfrac{2^r-1}{3^r}\right\}. \\
\text{For 4 equal arms:} & E_r^{\mathrm{IV}} = 4.2^{r-4}(r-1)!\left\{1 + \dfrac{2.3^r-2}{4^r}\right\}. \\
\text{For 5 equal arms:} & E_r^{\mathrm{V}} = 5.2^{r-5}(r-1)!\left\{1 + \dfrac{3.4^r+2.3^r-2.2^r-3}{5^r}\right\}. \\
\text{For many arms:} & E_r^{\infty} = r!.
\end{array}\right\} \quad (4)$$

The superscript attached to E_r indicates the number of arms in the chromosome set. The values of E_r for these different types of chromosome set have been tabulated in Table 1 up to $r = 24$. Calculations could be made for unequal arms if the probability that a break should occur in a given arm were known. In many *Drosophila* species there are four nearly equal arms, three or four being autosomal, a somewhat longer arm constituting the whole or part of the X, and usually a very small autosomal arm. Thus the values of E_r for an X-bearing spermatozoon will be fairly close to those calculated for five equal arms.

Also tabulated in Table 1 is $\frac{(2r)!}{r!\,2^r}$ which is the total number of arrangements, eucentric and dyscentric, of r breaks. For large values of r the ratio of E_r to the total number of arrangements is approximately $\frac{a}{2^a}\sqrt{\binom{\pi}{r}}$ (Fano, 1942).

We can also calculate the probability of an eucentric arrangement within an arm, e.g. within the X-chromosome of *Drosophila melanogaster*. Taking the probability of a break in the X-chromosome to be one-fifth of the probability of a break anywhere in the set, the probability that there will be l breaks in the X-chromosome out of a total of r breaks in the set is $\binom{r}{l}4^{r-l}5^{-r}$, where $\binom{r}{l} = \frac{r!}{l!\,(r-l)!}$. These l breaks can unite eucentrically in E_l^{I} ways without any part of the X-chromosome being translocated or any part of any other chromosome being united to it. One way is complete restitution, $(E_l^{\mathrm{I}} - 1)$ ways are inversions. The other $r-l$ breaks occur among the other four equal arms. They can unite eucentrically in E_{r-l}^{IV} ways (where E_{r-l}^{IV} may be read for any value of $(r-l)$ in Table 1). Hence the mean number of eucentric arrangements in which the X-chromosome suffers no structural change is

$$H_r^{\mathrm{V}} = 5^{-r}\sum_{l=0}^{r}\binom{r}{l}4^{r-l}E_{r-l}^{\mathrm{IV}}, \quad (5)$$

Table 1

r	$\frac{(2r)!}{r!\,2^r}$	$E_r^{I}=E_r^{II}$	E_r^{III}	E_r^{IV}	E_r^{V}	E_r^{∞}	H_r^{V}	I_r^{V}
0	1	1	1	1	1	1	1	0
1	1	1	1	1	1	1	1	0
2	3	2	2	2	2	2	1·64	0·04
3	$1·5 \times 10^{1}$	8	7·5556	7·25	7·04	6	4·584	0·152
4	$1·05 \times 10^{2}$	$4·8 \times 10^{1}$	$4·2667 \times 10^{1}$	$3·9 \times 10^{1}$	$3\,648 \times 10^{1}$	$2·4 \times 10^{1}$	$1·9278 \times 10^{1}$	0·5616
5	$9·45 \times 10^{2}$	$3·84 \times 10^{2}$	$3·2474 \times 10^{2}$	$2·8275 \times 10^{2}$	$2·5405 \times 10^{2}$	$1·2 \times 10^{2}$	$1·1022 \times 10^{2}$	2·6250
6	$1·0395 \times 10^{4}$	$3·84 \times 10^{3}$	$3·1289 \times 10^{3}$	$2·6025 \times 10^{3}$	$2·2456 \times 10^{3}$	$7·2 \times 10^{2}$	$8·0362 \times 10^{2}$	$1·6020 \times 10^{1}$
7	$1·3514 \times 10^{5}$	$4·608 \times 10^{4}$	$3·6567 \times 10^{4}$	$2·9188 \times 10^{4}$	$2·4218 \times 10^{4}$	$5·04 \times 10^{3}$	$7·1588 \times 10^{3}$	$1·2417 \times 10^{2}$
8	$2·0270 \times 10^{6}$	$6\,4512 \times 10^{5}$	$5·0264 \times 10^{5}$	$3·8714 \times 10^{5}$	$3\,0958 \times 10^{5}$	$4·032 \times 10^{4}$	$7·5552 \times 10^{4}$	$1·1785 \times 10^{3}$
9	$3·4459 \times 10^{7}$	$1·0322 \times 10^{7}$	$7·9424 \times 10^{6}$	$5·9359 \times 10^{6}$	$4·5877 \times 10^{6}$	$3·6288 \times 10^{5}$	$9·2291 \times 10^{5}$	$1·3269 \times 10^{4}$
10	$6·5473 \times 10^{8}$	$1·8579 \times 10^{8}$	$1·4176 \times 10^{8}$	$1·0336 \times 10^{8}$	$7·7453 \times 10^{7}$	$3·6288 \times 10^{6}$	$1·2815 \times 10^{7}$	$1·7292 \times 10^{5}$
11	$1·3749 \times 10^{10}$	$3·7159 \times 10^{9}$	$2·8191 \times 10^{9}$	$2·0149 \times 10^{9}$	$1·4688 \times 10^{9}$	$3·9917 \times 10^{7}$	$1·9934 \times 10^{8}$	$2·5587 \times 10^{6}$
12	$3·1623 \times 10^{11}$	$8·1750 \times 10^{10}$	$6·1785 \times 10^{10}$	$4·3464 \times 10^{10}$	$3·0924 \times 10^{10}$	$4·7900 \times 10^{8}$	$3·4330 \times 10^{9}$	$4·2345 \times 10^{7}$
13	$7·9059 \times 10^{12}$	$1·9620 \times 10^{12}$	$1·4791 \times 10^{12}$	$1·0276 \times 10^{12}$	$7·1584 \times 10^{11}$	$6·2270 \times 10^{9}$		
14	$2·1346 \times 10^{14}$	$5·1012 \times 10^{13}$	$3·8390 \times 10^{13}$	$2·6415 \times 10^{13}$	$1·8069 \times 10^{13}$	$8·7178 \times 10^{10}$		
15	$6\,1903 \times 10^{15}$	$1·4283 \times 10^{15}$	$1·0737 \times 10^{15}$	$7·3325 \times 10^{14}$	$4·9389 \times 10^{14}$	$1·3077 \times 10^{12}$		
16	$1·9190 \times 10^{17}$	$4·2850 \times 10^{16}$	$3·2186 \times 10^{16}$	$2·1854 \times 10^{16}$	$1·4529 \times 10^{16}$	$2·0923 \times 10^{13}$		
17	$6·3327 \times 10^{18}$	$1·3712 \times 10^{18}$	$1·0294 \times 10^{18}$	$6·9591 \times 10^{17}$	$4·5759 \times 10^{17}$	$3·5569 \times 10^{14}$		
18	$2·2164 \times 10^{20}$	$4·6621 \times 10^{19}$	$3·4989 \times 10^{19}$	$2·3573 \times 10^{19}$	$1·5359 \times 10^{19}$	$6·4024 \times 10^{15}$		
19	$8·2008 \times 10^{21}$	$1·6783 \times 10^{21}$	$1·2593 \times 10^{21}$	$8·4627 \times 10^{20}$	$5·4722 \times 10^{20}$	$1·2165 \times 10^{17}$		
20	$3·1983 \times 10^{23}$	$6·3777 \times 10^{22}$	$4·7847 \times 10^{22}$	$3·2091 \times 10^{22}$	$2·0621 \times 10^{22}$	$2·4329 \times 10^{18}$		
21	$1·3113 \times 10^{25}$	$2·5511 \times 10^{24}$	$1·9137 \times 10^{24}$	$1·2816 \times 10^{24}$	$8·1931 \times 10^{23}$	$5·1091 \times 10^{19}$		
22	$5·6386 \times 10^{26}$	$1·0715 \times 10^{26}$	$8·0370 \times 10^{25}$	$5·3764 \times 10^{25}$	$3·4225 \times 10^{25}$	$1·1240 \times 10^{21}$		
23	$2·5374 \times 10^{28}$	$4·7144 \times 10^{27}$	$3·5361 \times 10^{27}$	$2·3635 \times 10^{27}$	$1·4994 \times 10^{27}$	$2·5852 \times 10^{22}$		
24	$1·1926 \times 10^{30}$	$2·1686 \times 10^{29}$	$1·6266 \times 10^{29}$	$1·0865 \times 10^{29}$	$6·8730 \times 10^{28}$	$6·2045 \times 10^{23}$		

Table 2

αqD	S_1	S_2					S_3		$1-S_1/S_2$					S_3/S_2	
No. of arms...		1 or 2	3	4	5	Many	5	Many	1 or 2	3	4	5	Many	5	Many
0·32	1·337	1·357	1·357	1·357	1·357	1·356	0·3966	0·3952	0·0146	0·0145	0·0144	0·0143	0·0140	0·2923	0·2914
0·50	1 543	1·596	1·595	1·594	1·594	1·592	0·7000	0·6942	0·0330	0·0325	0·0322	0·0320	0·0309	0·4391	0·4360
0·72	1·811	1·932	1·929	1·928	1·926	1·920	1·171	1·152	0·0627	0·0615	0·0606	0·0601	0·0572	0·6080	0·5996
0·98	2·151	2·405	2·398	2·393	2·389	2 373	1·907	1·849	0·1056	0·1030	0·1011	0·0998	0·0935	0·7979	0·7793
1·28	2·577	3·078	3·060	3·047	3·038	2·996	3·068	2·915	0·1626	0·1576	0·1541	0·1517	0·1396	1·010	0·9731
1·62	3·107	4·053	4·010	3·980	3·959	3·858	4·933	4·553	0·2334	0·2250	0·2191	0·2150	0·1945	1·246	1·180
2·00	3·762	5·502	5·403	5 334	5·287	5·060	7·986	7·090	0·3162	0·3037	0·2947	0·2885	0·2565	1·510	1·401
2·88	5 557	11·21	10·75	10·43	10·21	9·172	21·86	17·29	0·5041	0·4831	0·4671	0·4556	0·3941	2·142	1·885
3·92	8·253	26·57	24·59	23·15	22·17	17·78	65·25	43·23	0·6894	0·6643	0·6435	0·6278	0·5357	2·943	2·432
5·12	12·29	74·67	66·03	59·60	55·22	36·82	218·2	112·2	0·8355	0·8139	0·7938	0·7775	0·6663	3·951	3·046
6·48	18·31	250·9	211·7	181·4	160·8	81·57	834·8	304·6	0·9270	0·9135	0·8991	0·8861	0·7755	5·190	3·734
8·00	27·31	1009	817·6	662 5	557·3	193·6	3718	870·8	0·9729	0·9666	0·9588	0·9510	0·8590	6·671	4·497

while the mean number of eucentric rearrangements in which the X-chromosome suffers inversion but not interchange with autosomes is

$$I_r^{\mathrm{V}} = 5^{-r} \sum_{l=2}^{r} \binom{r}{l} 4^{r-l} E_{r-l}^{\mathrm{IV}} (E_l^{\mathrm{I}} - 1). \tag{6}$$

H_r^{V} and I_r^{V} are tabulated in Table 1 for values of r up to 12.

Rearrangement after a given dose

If αD is the mean number of breaks primarily produced per nucleus by a dose D, then $e^{-\alpha D} (\alpha D)^r / r!$ is the proportion of nuclei having r breaks, and $e^{-\alpha D} (\alpha q D)^r / r!$ is the proportion of nuclei having r breaks none of which undergoes sister-union. The following results then follow from the preceding section:

The proportion of nuclei which are eucentric and without aberrations is

$$X = e^{-\alpha D} S_1, \quad \text{where} \quad S_1 = 1 + \alpha q D + \tfrac{1}{6} (\alpha q D)^2 + \ldots + \frac{(2\alpha q D)^r}{(2r)!} + \ldots. \tag{7}$$

The proportion of nuclei which are eucentric, with or without aberration, is

$$Y = e^{-\alpha D} S_2, \quad \text{where} \quad S_2 = 1 + \alpha q D + \tfrac{1}{3} (\alpha q D)^2 + \ldots + \frac{(2\alpha q D)^r}{(2r)!} E_r + \ldots. \tag{8}$$

The total number of primary breaks formed in eucentric nuclei, per total nucleus is

$$Z = e^{-\alpha D} S_3, \quad \text{where} \quad S_3 = \alpha q D + \tfrac{2}{3} (\alpha q D)^2 + \ldots + \frac{(2\alpha q D)^r}{(2r)!} E_r r + \ldots. \tag{9}$$

The series S_1 is the same for different chromosome sets and has the sum

$$S_1 = \cosh \sqrt{(2\alpha q D)}, \tag{10}$$

where $\cosh x = \frac{1}{2} (e^x + e^{-x})$ is the hyperbolic cosine.

The series S_2 and S_3 are different for chromosome sets containing different numbers of arms, and where necessary we attach a superscript to indicate the number of arms, e.g. S_2^{V} means the value of S_2 when E_r in formula (8) takes the values appropriate to five equal arms.

An algebraic expression for the sums S_2 and S_3 may be obtained in the case of a chromosome set with many arms. Defining

$$y = \sum_{r=1}^{\infty} \frac{(2x)^{2r} r!}{(2r)!}, \tag{11}$$

and differentiating (y/x) term by term (which is permissible since the series is absolutely convergent for all values of x), we obtain

$$\frac{d}{dx}\left(\frac{y}{x}\right) - 2y = 2,$$

whence

$$\frac{d}{dx} (e^{-x^2} y/x) = 2e^{-x^2}.$$

Integrating,

$$e^{-x^2} y/x = 2 \int_0^x e^{-x^2} dx = \sqrt{\pi} \operatorname{erf} x,$$

where $\operatorname{erf} x$, defined as $\dfrac{2}{\sqrt{\pi}} \displaystyle\int_0^x e^{-x^2} dx$, is the error function.

Thus
$$y = x\sqrt{\pi}\, e^{x^2} \operatorname{erf} x. \tag{12}$$
Evidently $S_2^\infty = 1 + y$, if $x = \sqrt{(\frac{1}{2}\alpha q D)}$. Hence
$$S_2^\infty = 1 + \sqrt{(\tfrac{1}{2}\pi\alpha q D)}\, e^{\frac{1}{2}\alpha q D} \operatorname{erf} \sqrt{(\tfrac{1}{2}\alpha q D)}. \tag{13}$$
By differentiating (11) we obtain
$$\sum_{r=1}^{\infty} \frac{(2x)^{2r} r!\, r}{(2r)!} = \tfrac{1}{2} x \frac{dy}{dx}$$
and by differentiating (12) we obtain
$$\tfrac{1}{2} x \frac{dy}{dx} = x^2 \left\{ 1 + \frac{1+2x^2}{x} \frac{\sqrt{\pi}}{2} e^{x^2} \operatorname{erf} x \right\}.$$
Inserting $x = \sqrt{(\frac{1}{2}\alpha q D)}$ we obtain
$$S_3^\infty = \tfrac{1}{2}\alpha q D \left\{ 1 + \frac{1+\alpha q D}{\sqrt{(\frac{1}{2}\alpha q D)}} \frac{\sqrt{\pi}}{2} e^{\frac{1}{2}\alpha q D} \operatorname{erf} \sqrt{(\tfrac{1}{2}\alpha q D)} \right\}. \tag{14}$$

Equations (10), (13) and (14) have been given by Lea & Catcheside (1945). Adequate tables are available of the exponential, hyperbolic cosine, and error functions (e.g. *W.P.A. Tables*, 1939–41), which are used to evaluate S_1, S_2^∞ and S_3^∞ in Table 2.

The evaluation of S_2 in the case of chromosome sets with a small number of arms is less convenient. In the case of a chromosome set with one or two arms we may proceed as follows. From equations (11) and (12) we have
$$\frac{y}{x} = \sum_{r=1}^{\infty} \frac{2^{2r} x^{2r-1} r!}{(2r)!} = \sqrt{\pi}\, e^{x^2} \operatorname{erf} x.$$
Integrating between $x = 0$ and $x = \sqrt{(\alpha q D)}$ we have
$$\sum_{r=1}^{\infty} \frac{(2\alpha q D)^r\, 2^{r-1} (r-1)!}{(2r)!} = \sqrt{\pi} \int_0^{\sqrt{(\alpha q D)}} e^{x^2} \operatorname{erf} x\, dx,$$
i.e.
$$S_2^{\mathrm{I}} = 1 + \sqrt{\pi} \int_0^{\sqrt{(\alpha q D)}} e^{x^2} \operatorname{erf} x\, dx, \tag{15}$$
where, as before, $\operatorname{erf} x$ denotes $\frac{2}{\sqrt{\pi}} \int_0^x e^{-x^2}\, dx$.

With the aid of equations (4) it would be possible to derive somewhat more complicated expressions for the values of S_2^{III}, S_2^{IV} and S_2^{V} involving the integral which occurs in equation (15). However, tables of this integral are not available and it has been found more convenient to compute S_2^{I}, S_2^{III}, S_2^{IV}, S_2^{V} and S_3^{V} by direct summation of the series given in equations (8) and (9) than to perform the integration numerically. The first ten terms of the series suffice for values of $\alpha q D$ up to unity, twenty terms are needed to give five significant figures for a value of $\alpha q D$ as high as 6. The results of the computation are set out in Table 2.

COMPARISON WITH EXPERIMENT

Experiments on the irradiation of *Drosophila* gametes provide suitable means of comparing the predictions of the theory with experiment. One of the observable quantities is the proportion of viable sperm which have chromosome aberrations. The theoretical expression for this proportion is $1 - X/Y = 1 - S_1/S_2$, which is listed in Table 2 as a function of $\alpha q D$. Lea and Catcheside found that the theoretical curve for a chromosome set with

many arms fitted the experimental observations on *Drosophila melanogaster* satisfactorily when $\alpha q = 0{\cdot}57$ per 1000 r. This curve is shown in Fig. 1 as curve A, together with theoretical curves (B and C) calculated for a chromosome set with five equal arms, which should be more appropriate for *Drosophila*. Curve B is computed with the same value $\alpha q = 0{\cdot}57$ as was employed in curve A, to indicate the magnitude of the error involved in using the many-arm formula instead of the 5-arm formula. A better fit of the five-arm formula to the experimental points at 4000 and 5000 r. is obtained by taking a somewhat smaller value of αq, namely, $\alpha q = 0{\cdot}52$ per 1000 r., and this value has been used in computing curve C.

A second observable quantity is $1 - Y = 1 - e^{-\alpha D} S_2$, the proportion of total sperm which are non-viable. (In comparing this formula with experimental results on imago emergence with *Drosophila melanogaster*, the latter have to be corrected for the proportion of unirradiated eggs which fail to develop into adult flies.) Lea & Catcheside (1945) found that the many-arm formula satisfactorily agreed with experiment if the value $\alpha = 0{\cdot}75$ per 1000 r. was assumed, together with $\alpha q = 0{\cdot}57$ already derived. This curve is reproduced in Fig. 2 as curve A, together with the theoretical curve (B) calculated from the five-arm formula with the same constants $\alpha = 0{\cdot}75$ and $\alpha q = 0{\cdot}57$ per 1000 r. Again the difference between the two theoretical curves is not very great. A better agreement of the five-arm formula with the experimental points at large doses is obtained by combining the value $\alpha q = 0{\cdot}52$ used in curve C of Fig. 1 with $\alpha = 0{\cdot}78$ per 1000 r., and this has been done in computing curve C of Fig. 2. The values of α and q best fitting the experimental results on the two theories are set out in Table 3.

Table 3

	Many-arm formulae	Five-arm formulae
α = mean number of primary breaks per sperm per 1000 r.	0·75	0·78
αq = mean number of primary breaks per sperm per 1000 r. which do not undergo sister-union	0·57	0·52
p = probability of a break undergoing sister-union	0·24	0·33
q = probability of a break not undergoing sister-union	0·76	0·67

The changes made in the constants by the replacement of the many-arm formulae by the more appropriate five-arm formulae are not sufficient to upset seriously the agreement which Lea & Catcheside found between the value of q deduced in this manner and the value ($q = 0{\cdot}74$) deduced from an analysis of sex-ratio distortion (Catcheside & Lea, 1945*b*).

In connexion with their suggestion that recessive lethals accompany a certain proportion of chromosome breaks (including breaks which restitute) Lea & Catcheside (1945) pointed out that the number of recessive lethals (in viable sperm) should increase with dose in the same manner as S_3/S_2, since S_3/S_2 is the mean number of primary breaks per viable sperm. Calculating S_3 and S_2 on the many-arm formulae they found that S_3/S_2 increased approximately as the 0·84 power of the dose in the interval 1500–6000 r., which is not in accord with the experimentally found rather exact proportionality of the number of sex-linked recessive lethals induced by radiation to the dose. However, when S_3 and S_2 are computed on the five-arm formulae (see Table 2), it is found that S_3/S_2 increases approximately as the 0·92 power of the dose between 1500 and 6000 r., making the discrepancy less serious.

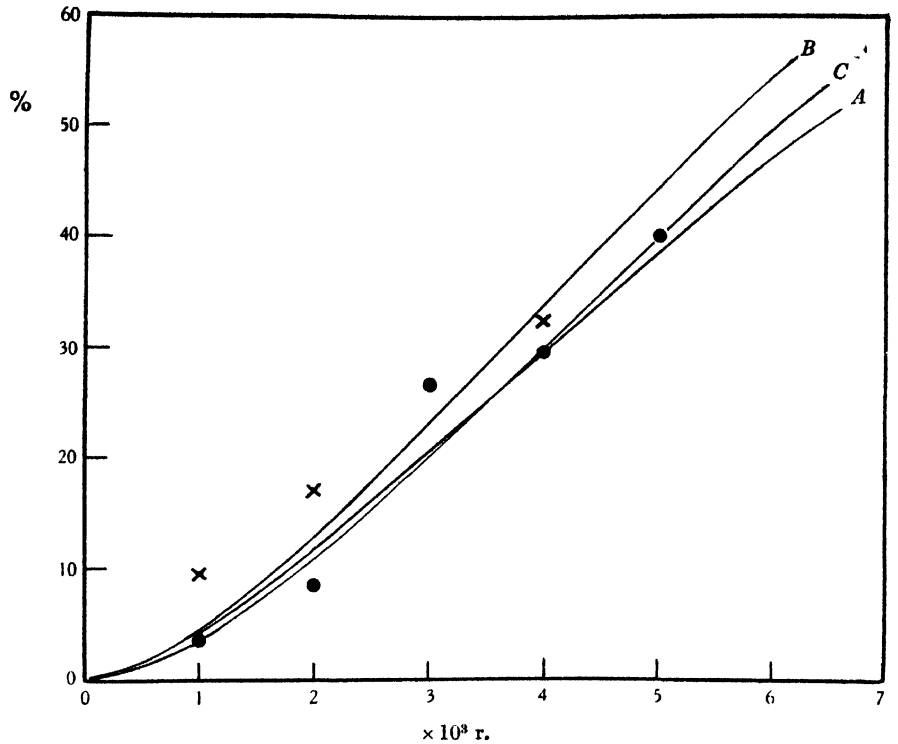

Fig. 1. Percentage of viable sperm having chromosomal structural change (curves theoretical, points experimental). ● Bauer, Demerec & Kaufmann (1938). × Catcheside (1938).

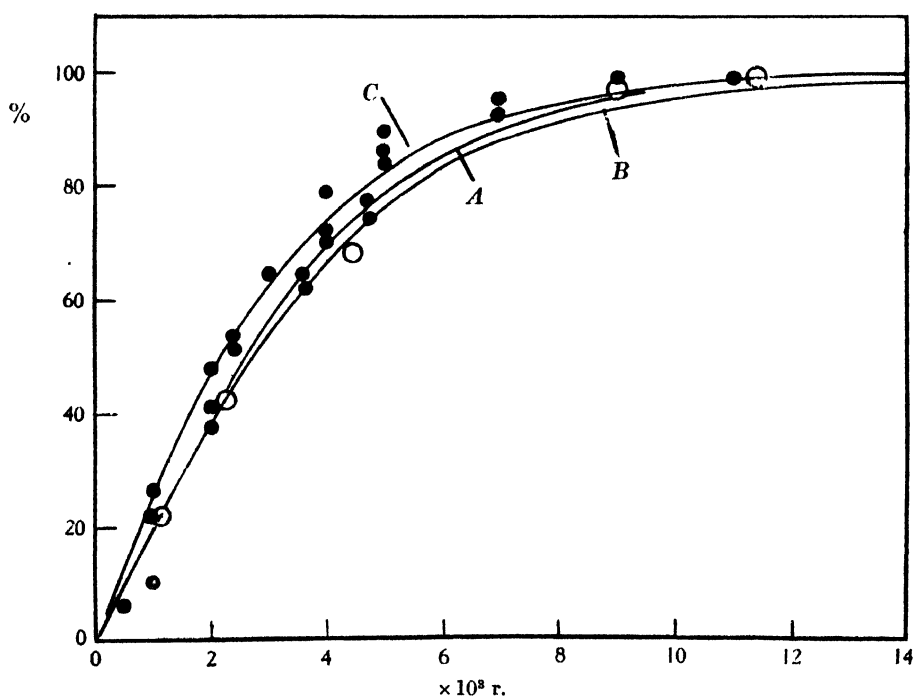

Fig. 2. Percentage of dominant lethals in *Drosophila melanogaster* sperm (curves theoretical, points experimental). ● Demerec & Fano (1944). ○ Catcheside & Lea (1945*a*).

The number of primary breaks per viable sperm (S_3/S_2) produced by a dose of 3000 r. Lea & Catcheside found to be 1·23, using the many-arm formulae with $\alpha q = 0{\cdot}57$ per 1000 r. When the calculation is repeated using the five-arm formulae and $\alpha q = 0{\cdot}52$ per 1000 r., the value is found to be 1·20. The agreement Lea & Catcheside found between estimates of the number of primary breaks per viable sperm based on the analysis of recessive lethals and of structural changes respectively is thus not upset by replacing their approximate calculations by the present more exact calculation.

Summary

A theory is given of the process of chromosomal structural rearrangement following irradiation. The theory applies to nuclei in which the union of breakage ends is at random, and assumes that the number of breaks primarily produced is proportional to the dose.

Formulae and tables are given enabling the proportion of nuclei undergoing eucentric and dyscentric types of rearrangement respectively to be calculated as a function of the dose, the cases of nuclei with 1, 2, 3, 4, 5 or many chromosome arms in the set being separately considered. The five-arm calculation is compared with published experimental results of the irradiation of the spermatozoa of *Drosophila melanogaster*.

REFERENCES

Bauer, H., Demerec, M. & Kaufmann, B. P. (1938). X-ray induced chromosomal alterations in *Drosophila melanogaster*. *Genetics*, **23**, 610.

Carlson, J. G. (1941). An analysis of X-ray induced single breaks in neuroblast chromosomes of the grasshopper (*Chortophaga viridifasciata*). *Proc. Nat. Acad. Sci., Wash.*, **27**, 42.

Catcheside, D. G. (1938). The effect of X-ray dosage upon the frequency of induced structural changes in the chromosomes of *Drosophila melanogaster*. *J. Genet.* **36**, 307.

Catcheside, D. G. & Lea, D. E. (1945*a*). The rate of induction of dominant lethals in *Drosophila melanogaster* sperm by X-rays. *J. Genet.* **47**, 1.

Catcheside, D. G. & Lea, D. E. (1945*b*). Dominant lethals and chromosome breaks in ring-*X* chromosomes of *Drosophila melanogaster*. *J. Genet.* **47**, 25.

Demerec, M. & Fano, U. (1944). Frequency of dominant lethals induced by irradiation in sperms of *Drosophila melanogaster*. *Genetics*, **29**, 348.

Fano, U. (1942). Mechanism of induction of gross chromosomal rearrangements in *Drosophila* sperms. *Proc. Nat. Acad. Sci., Wash.*, **29**, 12.

Fisher, R. A. & Yates, F. (1938). *Statistical Tables for Biological, Agricultural and Medical Research.* London: Oliver and Boyd.

Lea, D. E. & Catcheside, D. G. (1942). The mechanism of the induction by radiation of chromosome aberrations in *Tradescantia*. *J. Genet.* **44**, 216.

Lea, D. E. & Catcheside, D. G. (1945). The relation between recessive lethals, dominant lethals, and chromosome aberrations in *Drosophila*. *J. Genet.* **47**, 10.

Muller, H. J. (1940). An analysis of the process of structural change in chromosomes of *Drosophila*. *J. Genet.* **40**, 1.

Muller, H. J. (1941). Induced mutations in *Drosophila*. *Cold Spr. Harb. Symp.* **9**, 151.

Pontecorvo, G. (1942). The problem of dominant lethals. *J. Genet.* **43**, 295.

Sax, K. (1940). An analysis of X-ray induced chromosomal aberrations in *Tradescantia*. *Genetics*, **25**, 41.

Stadler, L. J. (1941). The comparison of ultraviolet and X-ray effects on mutation. *Cold Spr. Harb. Symp.* **9**, 168.

W.P.A. Mathematical Tables (1939–41). New York: Works Projects Administration.

NATURAL SELECTION IN EXPERIMENTS WITH POPULATION INVERSIONS

By N. P. DUBININ and G. G. TINIAKOV

(With Three Text-figures)

Dubinin & Tiniakov (1945–6) discovered that the karyotypes of urban and rural populations of *Drosophila funebris* are different.

Dobzhansky (1943), Dubinin, Sokolov & Tiniakov (1937), and Dubinin & Tiniakov (1945) described cyclic changes in inversion frequencies during the course of the year. All these researches give evident, though indirect, proof that natural selection acts on the karyotype of populations. The work here described is the first attempt at a direct proof of this very important principle.

Dubinin & Tiniakov (1945) found that the frequency of inversions in *D. funebris* is lowest in the spring after hibernation. In summer, during the period of reproduction, this frequency rises, and it reaches its maximum when the populations are in their prime. The inversion frequency falls in the winter. It is of great interest to discover which environmental factors cause the difference between the karyotypes of urban and rural populations, and the changes in inversion frequencies with the seasonal cycle.

We have investigated the changes occurring during hibernation. The flies hibernated in veneer boxes (30 × 20 × 20 cm.) with one glass side, and a hole for ventilation in one of the other sides. Each box contained 1000–5000 flies. During the winter of 1944–5 the boxes, containing a total of 22,512 flies, were kept in a cellar at temperatures from −2 to 3° C. The populations were examined cytologically before and after hibernation, in order to observe changes in the frequency of inversions due to natural selection.

The initial populations had three inversions, II-1, II-2 and IV-1. They remained in the cold for 1, 1½ and 2½ months. Table 1 and Fig. 1 show that the mortality is very heavy, and increases with the length of hibernation, till after 2½ months only 0·56% of flies

Table 1

Population	Duration of hibernation months	Initial no.	No. of survivors ♂	♀	Total	% survivors
Samoteka, Moscow, boxes 10, 11	1	5,100	171	176	347	6·80
Samoteka, Moscow, boxes 3, 6; Ivanovo, box 2	1½	5,960	83	70	153	2·57
Ivanovo, boxes 3, 4, 5	2½	11,452	35	29	64	0·56
Total		22,512	289	275	564	2·50

survived. Males and females survived in roughly equal numbers. Table 2 shows that in each population the frequency of individuals heterozygous for inversions II-1 diminished, while that of individuals heterozygous for IV-1 increased. The behaviour of heterozygotes for inversion II-2 is so far not clear. But it is clear that fitness, as shown by differential survival during hibernation, is increased by IV-1, and diminished by II-1.

It is very important that the results obtained in an experiment on hibernation agree with those on the changes of inversion frequency during the summer months. The frequency of inversion II-1 rises in summer, but falls in winter. Inversion IV-2 shows the opposite behaviour.

In this experiment the number of flies which survived hibernation and were studied cytologically is comparatively low (130). We therefore carried out a second experiment

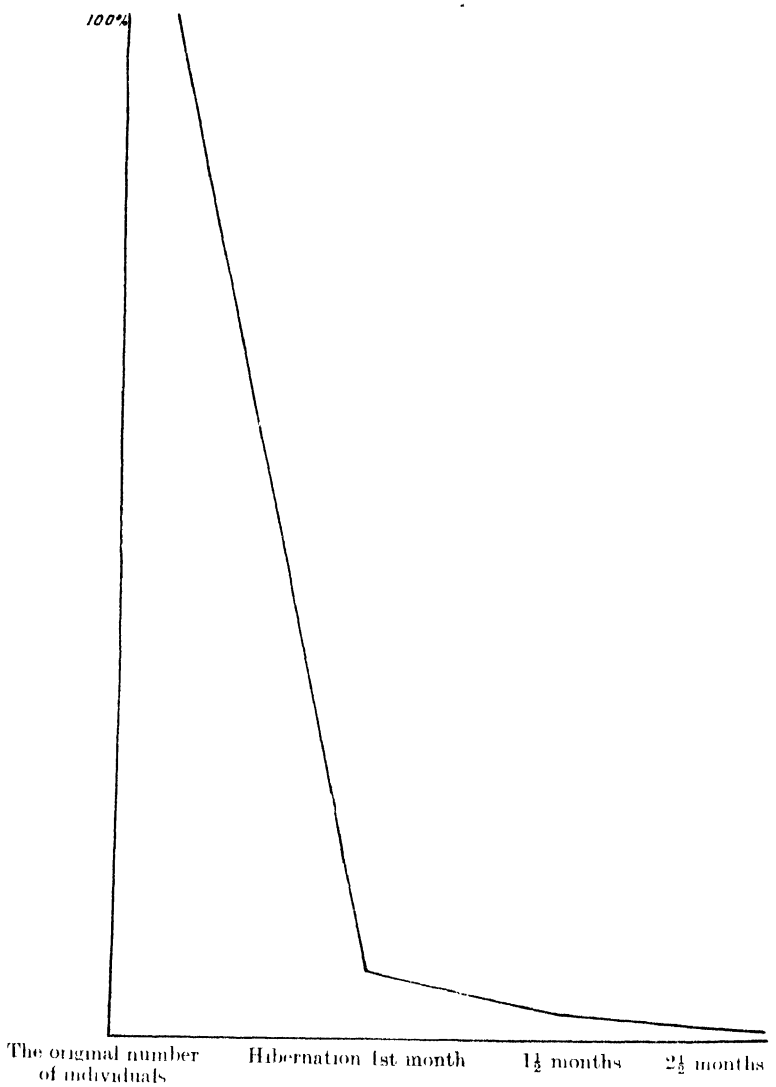

Fig. 1. Comparative viability during winter months.

Table 2

Population	Category	Non-hibernating controls No.	Non-hibernating controls %	Survivors of hibernation No.	Survivors of hibernation %
Ivanovo	Total	367	-	72	–
	Heterozygotes for inversion II-1	116	31·60	10	13 88
	,, ,, ,, II-2	6	1·63	2	2 77
	,, ,, ,, IV-1	20	5 44	6	8 31
Samoteka	Total	261	--	58	—
	Heterozygotes for inversion II-1	134	51 34	18	31·03
	,, ,, ,, II-2	70	26 93	10	17 24
	,, ,, ,, IV-1	7	2 69	13	22 41

with stocks specially bred for the purpose. One stock was homozygous for inversion II-4, another for the standard gene order, while the third was made by crossing these two, and

was therefore heterozygous for the inversion. These karyotypically different categories of flies were placed in boxes under conditions of hibernation and samples were taken after 15, 30 and 45 days. Fig. 2 and Table 3 show the results of the experiment.

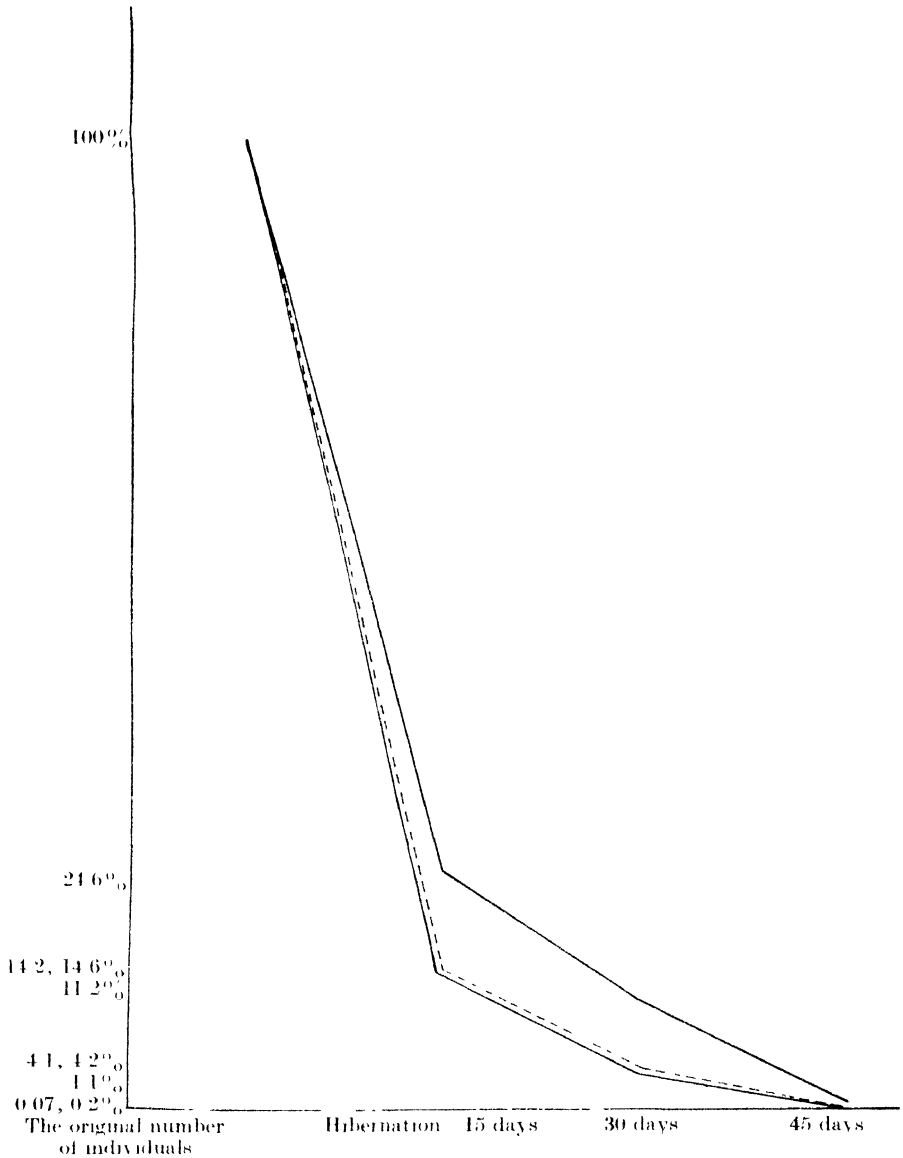

Fig. 2. Comparative viability of different genotypes during hibernation. The upper curve—the standard gene arrangement. The broken curve—homozygotes for the inversion No. 1. The lower curve—heterozygotes for the inversion No. 1.

Table 3. *Comparative survival of different karyotypes during hibernation*

Duration of hibernation days	Normal order			Heterozygotes for inversion II-1			Homozygotes for inversion II-1		
	Initial no.	No. of survivors	% survival	Initial no.	No. of survivors	% survival	Initial no.	No. of survivors	% survival
15	2000	593	24.6	2000	285	14.2	1847	270	14.1
30	3092	367	11.2	2318	94	4.1	3483	147	4.2
45	1092	12	1.1	1483	1	0.07	1471	3	0.2

The table and diagram show the enormous mortality of the flies during hibernation. Once more, and this time more clearly, we see the differential survival of different karyotypic categories. Flies with a normal karyotype survived better than either homozygotes or heterozygotes for the inversion. The degree of survival of the homozygotes and heterozygotes was equal. This speaks for the complete dominance of those genes which, being

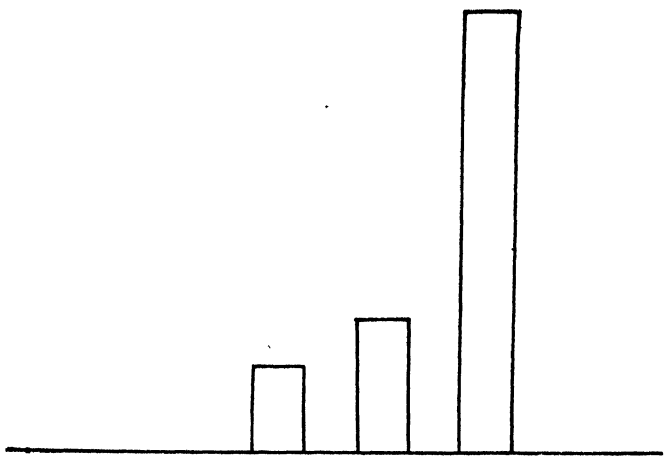

Fig. 3. The degree of viability of the normal karyotype as compared with inversions.

located in the inversion, determine their selective value. It will be very interesting to investigate the manifestation of those factors in homozygotes and heterozygotes, which determine the preferential increase of inversions in summer, and to ascertain whether they are due to a single group of genes which acquire a different selective value under different environmental conditions, or whether two different groups of factors are responsible, one for the selection in summer, the other for that in winter. Fig. 3, also derived from Table 3, shows the predominant survival of flies with a normal karyotype as compared with those with one or two chromosomes with an inverted order, as a function of length of hibernation. After 15 days the normal flies were 1·7 times as viable, after 30 days they were 2·6 times more viable, and after 45 days 8·5 times.

Conclusions

1. Our data show that the karyotypic differentiation of *Drosophila funebris* populations into urban and rural races, the diminution of inversion frequency in the towns destroyed by the war, and the changes in inversion frequencies with the months of the seasonal cycle, must all be regarded as vivid manifestations of the action of natural selection on the evolution of the karyotype.

2. Our material for the first time gives direct experimental evidence of the effect of natural selection on the karyotypic structure of populations. The experiments on hibernation show the differential survival of different karyotypic structures which are widespread in populations in nature. Different inversions react differently to the same experimental conditions.

3. This work has therefore discovered a new trend in the genetics of populations. It will become possible to apply natural selection as a method of direct experimental analysis for the solution of the several principal problems of the evolution of populations.

REFERENCES

DOBZHANSKY, TH. (1943). *Genetics*, **28**, 163–86.

DUBININ, N. P., SOKOLOV, N. N. & TINIAKOV, G. G. (1937). *Biol. Zh., Mosk.*, **6**, 1007–54.

DUBININ, N. P. & TINIAKOV, G. G. (1945). Seasonal cycles and the concentration of inversions in populations of *Drosophila funebris*. *Amer. Nat.* **79**, 785.

DUBININ, N. P. & TINIAKOV, G. G. (1946). Structural variability of chromosomes in urban and rural populations. *C. R. Acad. Sci. U.R.S.S.* **51**, 155.

STUDIES ON *STREPTOCARPUS*

II. COMPLEMENTARY SUBLETHAL GENES

By W. J. C. LAWRENCE, *John Innes Horticultural Institution*

(With Plates 1–4)

Object of study

The role of sublethality in speciation has recently become a matter of some speculation. In particular, attention has been focused on systems in which complementary genes could act as isolating mechanisms (Dobzhansky, 1937). A number of cases are on record in which it seems that the action of complementary genes could account for the production of lethal progeny in interspecific and intervarietal crosses.

Streptocarpus is yet another plant in which sublethals occur in certain crosses. The object of study has been to survey the whole of the genus to see if further information could be obtained respecting the role of sublethality in speciation.

Material

In the course of experiments on the inheritance of flower colour in the genus *Streptocarpus* (Lind.), it was noticed that seventeen out of thirty-one seedlings in a cross between *S. Rexii* (Hook) and a garden form (1/33) were abnormal. Growth was slow and distorted, and the abnormal seedlings died without progressing beyond the cotyledon stage. Experiments were made to see if there was a genetic basis for this abnormality (Pl. 1, fig. 1).

It will be necessary to give a brief description of normal and abnormal plants before proceeding to the experimental results.

(i) *Normal plants.* The very young *Streptocarpus* seedling has two equal cotyledons, but in the course of a few days one begins to grow rapidly, while the other ceases to grow and eventually dies (Pl. 1, fig. 2). A meristem at the base of the surviving cotyledon gives rise to an adventitious 'leaf'. This either engulfs the cotyledon or carries it on its tip. In the case of the unifoliate species of *Streptocarpus* this modified cotyledon is the only foliar organ developed. In the rosulate species a number of true leaves are also produced. In this account the term 'cotyledon' refers to the whole of the adventitious growth associated with the true cotyledon, while 'leaf' will be used only for true leaves.

(ii) *Abnormal plants.* The development of the surviving cotyledon by basal growth proceeds normally for the first few weeks. The onset of abnormality varies considerably and depends largely on cotyledon shape in the early seedling stage. In normal plants cotyledon width does not vary much, but in abnormals it ranges from little more than the width of the midrib to as wide as a normal cotyledon is at this stage (Pl. 1, fig. 3). In narrow-cotyledoned plants abnormality can be detected when the cotyledon is about 5 mm. long, whereas in broad-cotyledoned plants it may not be recognized until the cotyledon is 30–40 mm. in length (Pl. 2, fig. 1).

Usually abnormality is first indicated by the surface of the cotyledon becoming convex and assuming a dark, shiny green colour. As growth proceeds the characteristic venation of the normal cotyledon fails to appear and the laminal development is usually greatly

reduced and irregular (Pl. 2, fig. 2). The base of the cotyledon is attenuated instead of rounded. Irregular proliferation takes the place of regular production of true leaves. In short, the growing point is disorganized and the laminae reduced and contorted.

In its extreme form, the abnormal plant dies when the cotyledon is 5–10 mm. long. On the other hand, some abnormal plants produce an abundance of leaves (though the laminae are reduced and distorted), and occasionally may also bear normal flowers and seed (Pl. 2, fig. 3). All grades are found between these extremes, but usually the malformed cotyledon does not grow more than a few centimetres in length, only a few distorted leaves are produced, and no flowers. It appears to be a general rule that abnormality is confined to the leaf system and does not extend to the inflorescence.

Under natural conditions no abnormal plant would survive. This is clear from the fact that unless they are removed from the seed pan at an early stage, abnormals are speedily overgrown by their normal sibs and eliminated. Hence the abnormal plants might, in the Darwinian sense, be described as lethals. But when they are removed from the competition of their normal sibs, many of the abnormal plants live for months or even years (rosulate forms of *Streptocarpus* are perennial), therefore in this account they will be described as sublethals.

EXPERIMENTAL RESULTS

(1) *Varieties*

Normal plants, self-pollinated, always yield normal progeny. In cross-pollinations between normals, four types of F_1 are found: (1) all normal; (2) three-quarters normal, one-quarter sublethal; (3) half normal, half sublethal; and (4) all sublethal. Sublethal plants selfed usually give normals and sublethals. This breeding behaviour and that observed on backcrossing the F_1's to both parents are consistent with the hypothesis that the sublethal condition is due to the action of two complementary dominant sublethal genes, here designated **L** and **E**. Normals whose genotype is **LLee**, **Llee**, **llEE** or **llEe** are indistinguishable phenotypically from **llee** normals. Further, it will be seen from Tables 1 and 3 that the ratios of normals to sublethals are generally speaking in agreement with those expected on the assumption that **LLEE**, **LLEe**, **LlEe** and **LlEE** forms are indistinguishable from one another. Hence, in so far as the evidence goes, the genes **L** and **E** may properly be described as dominants.

The gene **L** was originally found in *S. Rexii* (Pl. 3, fig. 1) raised from seed collected in 1931 in the Knysna district of Cape Province, South Africa. The gene **E** was found in a garden form (1/33) raised from seeds supplied by a nurseryman. Seven other garden forms which proved to carry **E** were all derived from cross-pollinations with this one plant. Three of these seven plants were of the constitution **llEE** and were used in later crosses as testers for **L** (Pl. 3, fig. 2). *Rexii* proved to be **LLee** and was used as a tester for **E**. The results of the selfings and crossings made among garden forms are given in Table 1.

Mutation of **L** *and* **E**

When sublethal plants are grown on, occasionally an individual produces one or more leaves which, in part or whole, appear to be normal (Pl. 4, fig. 1). If a plant produces several of these normal leaves they will usually be found to arise from the same part of the plant, and since their growth is much more vigorous than that of the sublethal leaves, the normal portion, or rosette, may ultimately establish itself to the exclusion of the sublethal

Table 1

Family	Parents		Progeny	
			Normal	Sublethal
LLee selfed				
123/38	*Rexii*	—	Many	0
Llee selfed				
114/37	$66^{1}/36$	—	33	0
202/39	$128^{1}/38$	—	55	0
203/39	$128^{2}/38$	—	55	0
204/39	$128^{3}/38$	—	55	0
208/39	$66^{2}/36$	—	33	0
LLee × **Llee**				
137/38	*Rexii*	$66^{2}/36$	33	0
138/38	*Rexii*	$66^{1}/36$	33	0
205/39	*Rexii*	$128^{1}/38$	55	0
Llee × **LLee** (*Rexii*)				
139/38	$66^{2}/36$	*Rexii*	33	0
303/39	$128^{1}/38$	*Rexii*	120	0
304/39	$115^{23}/37$	*Rexii*	120	0
LLee × **llee**				
40/36	*Rexii*	$21^{11}/35$	50	0
39/36	*Rexii*	$21^{12}/35$	51	0
73/38	*Rexii*	$83^{1}/36$	25	0
225/39	*Rexii*	$92^{27}/36$	11	0
llee × **LLee**				
141/38	$21^{11}/35$	*Rexii*	33	0
llee × **llEE**				
33/42	$83^{1}/36$	$73^{3}/37$	20	0
llEe selfed				
13/34	1/33	—	128	0
65/38	1/33	—	50	0
llEe × **llEe**				
307/39	1/33	$116^{6}/37$	120	0
llEe × **llEE**				
332/39	1/33	$73^{29}/37$	60	0
llEe × **llee**				
35/36	1/33	$21^{11}/35$	31	0
211/39	1/33	$21^{11}/35$	33	0
37/36	1/33	$21^{12}/35$	34	0
169/40	1/33	$83^{1}/36$	40	0
304/40	1/33	$92^{27}/36$	100	0
llee × **llEe**				
107/37	$21^{12}/35$	1/33	33	0
152/40	$21^{11}/35$	1/33	50	0
303/40	$92^{27}/36$	1/33	100	0
llEE × **LLee** (*Rexii*)				
224/39	$26^{26}/36$	*Rexii*	0	20
302/39	$26^{26}/36$	*Rexii*	0	120
333/39	$73^{29}/37$	*Rexii*	0	60
LLee × **llEE**				
196/39	*Rexii*	$73^{3}/37$	0	20
329/39	*Rexii*	$26^{26}/36$	0	60

Table 1 (*cont.*)

Family	Parents		Progeny: Normal	Progeny: Sub-lethal	χ^2: Deviation	χ^2: Heterogeneity	D.F.	P
Llee × llEe (3 : 1)								
115/37	$66^{1}/36$	1/33	24	17				
117/37	$66^{2}/36$	1/33	30	9				
206/39	$128^{3}/38$	1/33	116	53				
Total			170	79	6·0094		1	0·02–0·01
						3·6412	2	0·2 –0·1
llEe × Llee (3 : 1)								
116/37	1/33	$66^{1}/36$	27	10				
145/38	1/33	$66^{2}/36$	81	39				
210/39	1/33	$128^{3}/38$	133	46				
258/39	1/33	$115^{23}/37$	60	20				
306/39	1/33	$115^{23}/37$	88	32				
Total			389	147	1·6816		1	0·2 –0·1
						2·8492	4	0·7 –0·5
llEe × LlEe (5 : 3)								
330/39	1/33	$128^{6}/38$	109	51				
331/39	1/33	$128^{6}/38$	121	59				
Total			230	110	3·8431		1	0·05
						0·5881	1	0·5 –0·3
LLee × llEe (1 : 1)								
66/36	*Rexii*	1/33	14	17				
126/37	*Rexii*	1/33	21	19				
298/39	*Rexii*	124/36	33	14				
Total			68	50	2·7458		1	0·1 –0·05
						5·3253	2	0·1 –0·05
llEe × LLee (1 : 1)								
118/37	1/33	*Rexii*	20	20				
128/38	1/33	*Rexii*	61	59				
74/38	$70^{61}/36$	*Rexii*	14	16				
226/39	$106^{37}/36$	*Rexii*	10	10				
301/39	$106^{37}/36$	*Rexii*	53	67				
227/39	$102^{39}/37$	*Rexii*	6	14				
305/39	$116^{8}/37$	*Rexii*	61	58				
Total			225	244	0·7697		1	0·5 –0·3
						1·4258	6	0·98–0·95
LlEe × LLee (1 : 1)								
323/39	$128^{5}/38$	*Rexii*	72	76	0·1081		1	0·8 –0·7
						—		
llEE × LlEe (1 : 1)								
344/39	$73^{29}/37$	$128^{6}/38$	54	86	7·3143		1	0·01
						—		
LlEe selfed (7 : 9)								
324/39	$128^{5}/38$	—	63	78				
343/39	$128^{7}/38$	—	68	92				
Total			131	170	0·0170		1	0·7 –0·5
						0·6294	1	0·5 –0·3

part. Thus what was originally a sublethal plant may become fully normal. We may conveniently distinguish such plants from those which have always been normal by the term 'reverted'. Very rarely, the cotyledon itself may revert (Pl. 4, fig. 2). The production of reverted leaves may occur as early as the first leaf or not until years later, after a great many abnormal leaves have been produced.

These facts about reverted plants suggest that they arise by somatic mutation of the gene **L** to its recessive allele in plants heterozygous for **L** and carrying **E**; or conversely, by

mutation of **E** in plants heterozygous for **E** and carrying **L**. This hypothesis can be tested in several ways.

First, reverted plants of known parentage can be selfed: their progeny should consist of normals only. Seventeen plants were thus tested and all but one ($128^7/38$, which gave 299 normals to 1 sublethal) gave nothing but normals (Table 2). The one sublethal obtained

Table 2. *Reverted plants selfed*

Reverted plants	Constitution from parentage	Progeny	
		Normal	Sublethal
$128^7/38$	**LlEe**	299	1
$128^{10}/38$	**LlEe**	100	0
$128^9/38$	**LlEe**	170	0
$330^1/39$	**LlE(e)**	200	0
$330^2/39$	**LlE(e)**	15	0
$330^3/39$	**LlE(e)**	120	0
$330^4/39$	**LlE(e)**	200	0
$330^5/39$	**LlE(e)**	50	0
$330^6/39$	**LlE(e)**	150	0
$330^7/39$	**LlE(e)**	200	0
$331^2/39$	**LlE(e)**	100	0
$331^3/39$	**LlE(e)**	200	0
$331^4/39$	**LlE(e)**	200	0
$344^1/39$	**LlE(e)**	200	0
$346^3/39$	**L(l)E(e)**	200	0
$388^{18}/40$	**L(l)E(e)**	100	0
$383^{24}/40$	**L(l)E(e)**	100	0

from selfing $128^7/38$ is evidently the result of mutation. The case of $128^7/38$ is particularly informative, since it was possible to self it both before (68 normal: 92 sublethals) and after (299 normal: 1 sublethal) it had reverted. It is not possible, of course, to determine from selfings which gene has mutated. For comparison, the results from selfing six sublethal plants are given in Table 3. Three of them were known from their parentage to be **LlEe**

Table 3. *Sublethals selfed*

Sublethal plants	Constitution from parentage		Progeny	
			Normal	Sublethal
$128^5/38$	**LlEe**		87	104
$128^7/38$	**LlEe**		68	92
$329^1/39$	**LlEe**		21	29
		Total	176	225
$343^1/39$	?		24	25
$343^2/39$	?		25	23
$346^1/39$	?		4	9

types and gave the expected ratio of 9 sublethals to 7 normals. The constitutions of the other three were unknown, but in two cases the ratios obtained were significantly nearer 9 : 7 than 3 : 1.

Secondly, reverted plants from families segregating normal and sublethals, and reverted plants from families giving sublethals only, can be crossed to the test plants and the mutant gene identified. The second of these two methods is the more rigorous test, since the possibility of a normal but ungerminated seed being moved with the soil when pricking-off is in progress, later to germinate and appear to be part of the young plant, is entirely prohibited. The data are given in Table 4. In six cases **L** has mutated to **l**, in five cases **E** to **e**, and in one case ($329^1/39$) **L** has mutated to **l** and **e** to **E**. Thus, as far as the data go, it seems that the genes **L** and **E** mutate with approximately equal frequency

to the recessive condition. Mutation to the dominant has been observed in the garden forms of *Streptocarpus* on three occasions only, namely, in $329^1/39$, mentioned above, and in $69^4/42$ and $187^1/42$ selfed (Table 4). In each of the latter families there was one sublethal in 300 plants, and these two individuals were sublethal from the start, i.e. the

Table 4

Reverted to normal	Constitution from parentage	Crossed to **LLee**		Crossed to **llEE**		Selfed		Constitution from progeny	Direction of mutation
		Normal	Sub-lethal	Normal	Sub-lethal	Normal	Sub-lethal		
(*a*) $128^6/38$	**LlEe**	240	0	54	86	—	—	**Llee**	**E** to **e**
(*a*) $330^7/39$	**LlE(e)**	400	0	122	102	200	0	**Llee**	**E** to **e**
(*a*) $331^3/39$	**LlE(e)**	400	0	166	234	200	0	**Llee**	**E** to **e**
(*a*) $346^3/39$	**L(l)E(e)**	400	0	0	200	200	0	**LLee**	**E** to **e**
(*b*) $187^1/42$	**LlEe**	150	0	47	53	299	1	**Llee**	**E** to **e**
(*a*) $343^3/39$	**L(l)E(e)**	96	104	100	0	—	—	**llEe**	**L** to **l**
(*a*) $344^1/39$	**LlE(e)**	186	178	200	0	200	0	**llEe**	**L** to **l**
(*a*) $344^2/39$	**LlE(e)**	37	8	14	0	—	—	**llEe**	**L** to **l**
(*b*) $69^1/42$	**LlEe**	232	268	100	0	300	0	**llEe**	**L** to **l**
(*b*) $69^3/42$	**LlEe**	94	106	200	0	202	0	**llEe**	**L** to **l**
(*b*) $69^4/42$	**LlEe**	65	35	100	0	299	1	**llEe**	**L** to **l**
(*a*) $329^1/39$	**LlEe**	0	200	200	0	21	29*	**llEE**	**L** to **l** **e** to **E**

* From selfing sublethal portion of plant.
(*a*) Reverted plants from families segregating normals and sublethals.
(*b*) Reverted plants from families segregating sublethals only.

mutations occurred very early in the life of the plant, or possibly in a gamete. As will be seen from the constitution of $69^4/42$, the mutation was probably from **l** to **L** whereas in $187^1/42$ it was probably **e** to **E**. Similar mutations to the dominant have been observed in species crosses and are discussed under that heading.

An alternative explanation to the above would be that these sublethals arose from small portions of unmutated (sublethal) tissue in the reverted parents. This is perhaps unlikely, since, owing to the rarity of the sublethal seedlings (of the order 1 : 300 normals), it must be supposed that very small portions of sublethal tissue could persist for many months in an otherwise normal plant (cf. $69^2/42$ below).

The rate of mutation from dominant to recessive is determined, to some extent, by the genotypic background of the plant. Thus mutation (i.e. reversion) is much more common in some families than in others (Table 5). It has not been possible to correlate mutation frequency with environmental influences of any kind.

In all the facts so far presented, the evidence has been clear and coherent. One anomalous case has now to be discussed. This is the reverted plant $69^2/42$. Morphologically, $69^2/42$, after the original part of it had reverted, still bore a remnant of small sublethal leaves. The number and vigour of these varied somewhat in the first months after reversion, but eventually the normal portions of the plant completely suppressed the sublethal, and to all outward appearances the plant was indistinguishable from the normal condition. $69^2/42$ was self-pollinated thirteen times, seven pollinations between January and March, and five pollinations* in July 1943. Of the seven pollinations, three were made on flowers borne on fully normal rosettes, and four on rosettes which were mainly normal. The July pollinations were made after $69^2/42$ had become fully normal in appearance. The

* One other pollination gave highly anomalous results and is discussed later.

results from all these pollinations are given in Table 6. It will be seen that the three early pollinations involving normal rosettes are consistent in giving a large majority of normals, in all, 217 normals to 75 sublethals (2·9 : 1). In contrast, the four early pollinations involving mainly normal rosettes, gave a total of 268 normals to 131 sublethals (2·05 : 1). Finally, the five late pollinations gave a total of 233 normals: 267 sublethals (1 : 1·1).

Table 5

Family	Parents	Constitution of parents	Progeny: Normal	Progeny: Sublethal	Proportion of sublethals reverted
128/38	1/33 × *Rexii*	**llEe** × **LLee**	61	59	2 in 19 months; 2 more in 60 months out of possible 5
324/39	$128^{5}/38$ selfed	**LlEe**	63	78	0 in 10 months
323/39	$128^{5}/38$ × *Rexii*	**LlEe** × **LLee**	72	76	0 in 10 months
330/39	1/33 × $128^{6}/38$	**llEe** × **Llee***	109	51	44 in 7 months
344/39	$73^{29}/37$ × $128^{6}/38$	**llEE** × **Llee***	54	86	19 in 8 months
343/39	$128^{7}/38$ selfed	**LlEe**	29	51	3 in 8 months
346/39	$128^{7}/38$ selfed	**LlEe**	39	41	14 in 8 months
331/39	1/33 × $128^{8}/38$	**llEe** × **LeEe**	121	59	35 in 7 months
69/42	$26^{26}/36$ × $346^{3}/39$	**llEE** × **LLEe***	0	200	3 in 7 months
129/42	$346^{3}/39$ × $73^{3}/37$	**LLEe*** × **llEE**	0	200	0 in 7 months
334/43	*Rexii* × $73^{29}/37$	**LLee** × **llEE**	0	262	0 in 8 months
333/39	Reciprocal	**llEE** × **LLee**	0	60	0 in 10 months
333/43	$73^{3}/37$ × *Rexii*	**llEE** × **LLee**	1	179	0 in 8 months
336/43	$26^{26}/36$ × *Rexii*	**llEE** × **LLee**	1	99	1 in 9 months
329/39	Reciprocal	**LLee** × **llEE**	0	60	1 in 13 months
335/43	Reciprocal	**LLee** × **llEE**	0	100	0 in 9 months

* I.e. after reversion.

Table 6. $69^{2}/42$ *selfed*

Family	Raised from: Pollinations made	Raised from: Flowers borne on	Normals		Sublethals	
655/42	Jan.–Mar.	Normal rosette	69	**217**	31	**75**
31/43	Jan.–Mar.	Normal rosette	65		27	
37/43	Jan.–Mar.	Normal rosette	83		17	
33/43	Jan.–Mar.	Mainly normal rosette	53	**268**	47	**131**
42/43	Jan.–Mar.	Mainly normal rosette	65		35	
36/43	Jan.–Mar.	Mainly normal rosette	74		26	
39/43	Jan.–Mar.	Mainly normal rosette	76		23	
319/43	July	Normal rosette	*c.* 6		*c.* 94*	
320/43	July	Normal rosette	48	**233**	52	**267**
321/43	July	Normal rosette	37		63	
322/43	July	Normal rosette	57		43	
323/43	July	Normal rosette	49		51	
324/43	July	Normal rosette	42		58	
Total			**724**		**567**	

* See p. 23.

Calculation of the heterogeneity between and within these three groups shows that it is greater between than within them:

Items	χ^2	Degrees of freedom	Mean square	Variance ratio	Probability
Between groups	71·06	2	35·53	11·14	0·01–0·001
Within groups	28·76	9	3·19	—	—
1st group	6·08	2	3·04	—	0·05–0·02
2nd group	15·53	3	5·18	—	0·01–0·001
3rd group	9·21	4	2·30	—	0·10–0·05

Taken together, the above results strongly suggest that $69^2/42$ is a chimaera of normal and sublethal tissues. At the time of the early pollinations, it seems that $69^2/42$ was a mericlinal chimaera, in which apparently normal rosettes were composed of a higher proportion of normal tissue than were the mixed rosettes. By the time the late pollinations had been made, $69^2/42$ seems to have become a periclinal chimaera of one layer of normal over sublethal tissue. The numbers obtained from self-pollination are close to the 7 normal : 9 sublethal ratio calculated for a sublethal plant of constitution **LeEe** (the expected genotype for $69^2/42$ before it had reverted).

That $69^2/42$ is actually a chimaera is supported by the results of propagating it from leaf cuttings. Three different leaves were used, the method being to cut them transversely into 1 in. sections, the proximal sides of which were placed in damp sand. Adventitious buds are produced just beneath the upper surface of the thicker veins, especially the midrib. Five plants were thus obtained from each of the three leaves, giving fifteen plants in all. Thirteen of these were fully normal in appearance: the other two bore sublethal portions (Pl. 4, fig. 3). We may assume, therefore, that somatic mutation in $69^2/42$ gave rise to mixed normal and sublethal tissues, which progressively sorted out until, finally, the plant became stabilized as a periclinal chimaera of normal over sublethal tissues.

Further evidence of irregularity and disorganization in somatic cells* was provided by a thirteenth family derived from the selfing of $69^2/42$. This family consisted of 94 sublethals and 6 normal plants. Five of these normals had curiously thick leaves and flowers, somewhat larger than other $69^2/42$ individuals. Chromosome counts on the root tips from three of these five plants showed that one of them was definitely tetraploid ($2n=64$), and two others probably so. The sixth individual with leaves and flowers of normal size and thickness was a diploid ($2n=32$). These tetraploids are the first recorded in garden *Streptocarpus*, and among the 50,000 or so plants raised at Merton during the last 10 years, only one other thick-leaved individual has been observed. Thus, the simultaneous occurrence of the tetraploids can almost certainly be correlated with the same somatic disturbances that gave rise to $69^2/42$.

(2) *Species*

The fact that certain plants of *Streptocarpus* carry dominant complementary sublethal genes seemed of sufficient interest to warrant a general search being made for them among twenty-three species, other than *Rexii* (see below), grown at Merton. Crosses were made therefore between each species and the **LLee** and **llEE** test plants, at least fifty plants being raised where possible. In most cases reciprocal crosses were made and more than one plant of each species was tested. In addition, three stocks of *Rexii* from widely separated localities were tested, namely, from Kirstenbosch Botanic Garden ($\mathbf{R^{Ki}}$), Stutterheim ($\mathbf{R^{S}}$), and Balfour ($\mathbf{R^{B}}$); also a fourth form, 'near *Rexii*', from East London ($\mathbf{R^{L}}$).

Of the twenty-three species tested (Tables 7–11), three failed to give seed with either tester, five gave F_1's with the **llEE** test plants only, while sixteen gave F_1's with both **LLee** and **llEE** test plants. Species homozygous for **L** or **E** would be expected to give all

* Irregularity of cell division in the growing-point is, in a somewhat different sense, characteristic of the growth of sublethals in *Streptocarpus*. The grotesque malformations of the different leaves on the same sublethal plant (e.g. Pl. 2, fig. 3) make this abundantly clear. Differences of leaf shape and size between different sublethal plants may be due to leaf-modifying genes, but this cannot apply to a given plant.

lethal progeny when crossed with the appropriate test plant: similarly, species heterozygous for **L** or **E** would be expected to give progeny approximately half of which were normal and half sublethal. No such F_1's were found, therefore all of the species tested may be assumed to be **llee** in constitution.

F_1's with **LLee** and **llEE**	F_1's with **llEE**	Crosses failed
Comptonii	*Galpinii*	*Daviesii*
cyaneus	*Haygarthii*	*gracilis*
Dunnii	*montigena*	*Pole-Evansii*
Eylesii	*pusillus*	
Gardenii	*Wilmsii*	
grandis		
insignis		
Johannis		
Meyeri		
Baudertii		
Michelmorei		
parviflorus		
Polackii		
polyanthus		
Vandeleuri		
Wendlandii		

The F_1's from the *Rexii* stocks must be considered separately. $\mathbf{R}^{K}$, $\mathbf{R}^{S}$ and $\mathbf{R}^{L}$ gave nothing but normals. Three plants of $\mathbf{R}^{B}$ were tested: two gave normals only, but the third yielded approximately half normals and half sublethals (217 : 183), thereby showing that this plant was **llEe**.

Table 7

Parents	Progeny: Normal	Progeny: Sublethal
LLee × *Rexii*^{K1}	253	0
Johannis	324	0
Baudertii	120	0
Michelmorei	100	0
Eylesii	200	0
Total	997	0
Rexii^{K1} × **LLee**	33	0
Rexii^{S}	20	0
Total	53	0

Parents	Progeny: Normal	Progeny: Sublethal
llEE × *Rexii*^{K1}	33	0
Rexii^{S}	100	0
Rexii^{L}	133	0
Polackii	220	0
pusillus	64	0
Meyeri	250	0
Baudertii	100	0
Haygarthii	2	0
Comptonii	33	0
grandis	125	0
Michelmorei	40	0
Wilmsii	19	0
montigena	100	0
Total	1219	0
Rexii^{K1} × **llEE**	33	0
Rexii^{S}	60	0
Gardenii	33	0
polyanthus	16	0
Galpinii	12	0
Vandeleuri	100	0
Total	254	0
Grand total	2523	

Now the **LLee** test plant, it will be remembered, is also a *Rexii* stock, hence the genes **L** and **E** have been found only within the species *Rexii*. Both the *Rexii* and $\mathbf{R}^{B}$ plants were raised from seeds gathered directly from the wild. The genes **L** and **E** therefore occur in wild populations of *Rexii* in the Cape Province.

As no species, outside *Rexii*, had been found to carry **L** or **E**, further crosses were made to see if inbreeding revealed the existence of genes concerned with the expression of lethality. Certain F_1's were backcrossed reciprocally and also crossed to the appropriate test plants (Tables 8–11).

Reciprocal crosses were made because it had already been established (Lawrence, unpublished) that cytoplasmic inheritance is common in *Streptocarpus*, hence it was clearly desirable to ascertain if cytoplasm type had any influence on the action of **L** and **E**.

Table 8

	LLee × species		Backcross to ♀		Backcross to ♂		F_1 (♀) × **llEE**		F_1 (♂) × **llEE**	
Species	Normal	Sub-lethal	Normal	Sub-lethal	Normal	Sub-lethal	Normal	Sub-lethal	Normal	Sub-lethal
Rexii[S]	280	0	—	—	200	0	111	89	52	48
Rexii[L]	268	0	—	—	200	0	104	91	106	86
insignis	200	0	—	—	196	0	—	—	109	91
Polackii	50	0	—	—	200	0	102	98	89	111
cyaneus	159	1	200	0	200	0	51	49	72	128
parviflorus	200	0	—	—	100	0	105	95	72	128
Gardenii	68	0	100	0	300	0	—	—	213	287
Meyeri	60	0	—	—	100	0	91	109	48	52
polyanthus	68	0	—	—	200	0	91	59	88	112
Comptonii	60	0	100	0	100	0	135	65	63	38
grandis	200	0	—	—	200	0	49	51	—	—
Dunnii	117	0	—	—	47	0	41	31	28	22
Vandeleuri	100	0	—	—	100	0	66	31	—	—
Wendlandii	44	0	—	—	100	0	194	106	160	240
Baudertii	100	0	200	0	—	—	96	104	88	112
Total	1974	1	600	0	2243	0	1236	978	1188	1455

Table 9

	Species × **LLee**		Backcross to ♀		Backcross to ♂		F_1 (♀) × **llEE**		F_1 (♂) × **llEE**	
Species	Normal	Sub-lethal	Normal	Sub-lethal	Normal	Sub-lethal	Normal	Sub-lethal	Normal	Sub-lethal
Rexii[L]	53	0	—	—	200	0	218	202	99	101
insignis	100	0	—	—	400	0	111	89	94	106
Polackii	150	0	200	0	100	0	51	49	81	119
cyaneus	230	0	—	—	200	0	106	94	94	106
parviflorus	1	0	—	—	200	0	78	72	43	57
Gardenii	53	0	200	0	200	0	105	95	88	112
Meyeri	43	0	—	—	200	0	106	94	77	123
grandis	100	0	198	0	200	0	29	21	60	90
Michelmorei	20	0	—	—	50	0	5	5	87	83
Dunnii	26	0	—	—	3	0	37	63	—	—
Vandeleuri	50	0	—	—	100	0	—	—	—	—
Wendlandii	129	0	100	0	96	0	200	100	—	—
Total	955	0	698	0	1949	0	1046	884	723	897

Table 10

	llEE × species		Backcross to ♀		Backcross to ♂		F_1 (♀) × **LLee**		F_1 (♂) × **LLee**	
Species	Normal	Sub-lethal	Normal	Sub-lethal	Normal	Sub-lethal	Normal	Sub-lethal	Normal	Sub-lethal
insignis	299	1	—	—	398	0	151	149	210	190
cyaneus	80	0	200	0	200	0	—	—	89	111
parviflorus	100	0	—	—	200	0	103	97	85	115
Gardenii	120	0	—	—	5	0	86	64	111	89
Dunnii	60	0	111	0	—	—	6	15	—	—
Wendlandii	91	0	200	0	200	0	26	40	159	141
Johannis (Brit.)	200	0	200	0	—	—	95	105	—	—
Vandeleuri	200	0	5	0	—	—	27	1	—	—
Eylesii	100	0	—	—	—	—	3	0	—	—
Total	1250	1	716	0	1003	0	497	471	654	646

As far as the expression of lethality is concerned, no cytoplasmic effect has been observed, the products of reciprocal crossing being alike. From 7597 F_1 plants raised by crossing twenty species with **LLee** and **llEE** test plants, only three sublethals were obtained. These

were from **LLee** × *cyaneus*; **llEE** × *insignis*; and *Wendlandii* × **llEE**. From 8759 B_1 plants, no sublethals were obtained. Considering the F_1 and B_1 data together, out of 16,456 plants, three were sublethals: one from *cyaneus*, one from *Wendlandii* and one from *insignis* crosses. It seems probable, therefore, that the occurrence of sublethals in these species crosses is fortuitous and not correlated with any particular recognizable factor or condition.

Table 11

	Species × **llEE**		Backcross to ♀		Backcross to ♂		F_1 (♀) × **LLee**		F_1 (♂) × **LLee**	
Species	Normal	Sub-lethal	Normal	Sub-lethal	Normal	Sub-lethal	Normal	Sub-lethal	Normal	Sub-lethal
Rexii^{L}	133	0	400	0	—	—	174	226	187	213
insignis	100	0	200	0	—	—	109	91	96	104
Polackii	160	0	200	0	600	0	83	117	207	293
cyaneus	120	0	200	0	200	0	196	204	85	115
grandis	130	0	100	0	—	—	120	80	103	97
Wendlandii	149	1	150	0	—	—	—	—	—	—
montigena	100	0	—	—	100	0	—	—	—	—
Total	892	1	1250	0	900	0	682	718	678	822

Table 12

		Normal	Sublethal
313/39	*Rexii*$^{S\,(No\ 2)}$ × 167^{1}/38 (*Rexii*$^{S\,(No\ 2)}$ × *Rexii*$^{S\,(No.\ 1)}$)	58	2
50/40	2^{29}/38 (*Wendlandii* × *grandis*) × *Wendlandii*	14	1
58/40	70^{61}/36 × 121^{1}/39 (73^{3}/37 × *Wendlandii*)	39	1
76/40	14^{6}/39 selfed (*Rexii* × *Wendlandii*)	26	2
307/43	*Polackii* D5 selfed	49	1
308/43	*Polackii* D5 × 73^{3}/37	99	1
346/43	*Polackii* D5 × *Rexii*	98	2
315/43	*Polackii* D11 × *Rexii*	99	1
Total		482	11

Table 13

	Normal	Sublethal		Normal	Sublethal
		216/43 (*Rexii* (**LLee**) × *Rexii*^{K1} (**llee**))			
216^{1}/43 × **llEE**	45	54	**llEE** × 216^{1}/43	—	—
216^{2}/43 × **llEE**	46	53	**llEE** × 216^{2}/43	56	44
216^{3}/43 × **llEE**	58	38	**llEE** × 216^{3}/43	62	34
				40	58
216^{4}/43 × **llEE**	56	42	**llEE** × 216^{4}/43	43	38
				46	48
216^{6}/43 × **llEE**	38	58	**llEE** × 216^{6}/43	45	46
	243	245		292	268
		217/43 (*Rexii*^{K1} (**llee**) × *Rexii* (**LLee**))			
217^{1}/43 × **llEE**	57	41	**llEE** × 217^{1}/43	46	49
	8	9			
217^{2}/43 × **llEE**	50	45	**llEE** × 217^{2}/43	46	52
217^{3}/43 × **llEE**	55	45	**llEE** × 217^{3}/43	53	35
217^{4}/43 × **llEE**	49	51	**llEE** × 217^{4}/43	53	45
	48	42		48	50
217^{5}/43 × **llEE**	49	49	**llEE** × 217^{5}/43	37	37
217^{6}/43 × **llEE**	43	40	**llEE** × 217^{6}/43	—	—
Total	359	322		283	268

In addition to the above three sublethal individuals, eleven have appeared (nine families) among the many thousands of plants raised for various genetical purposes (Table 12).

Finally, from the self-pollination of sixteen species and four *Rexii* stocks, not a single sublethal plant appeared in 12,765 progeny (including 5621 plants from the selfing of *Rexii* (**LLee**)).

The simplest explanation for the occurrence of these sporadic sublethal plants is that the genes **l** and **e** occasionally mutate to their dominant alleles. This view is supported by the examples mentioned on p. 21. In short, the genes **L–l** and **E–e** are somewhat unstable genes whose mutation rates appear to be similar or, at least, not widely different. It may not be without significance that no mutation was observed among over 5000 plants from selfing *Rexii* (**LLee**), whereas in hybrids between *Rexii* and various garden plants carrying **E**, the rate of mutation is quite appreciable. Increase of mutation in hybrids has been observed on several occasions in *Streptocarpus* where flower colour is concerned.

Certation

(1) *Specific.* If the genes **L** and **E** are concerned in speciation, then it is desirable to know whether the rate of growth of pollen tubes carrying **L** and **E** is different as between **L** and **E** styles. Accordingly *Rexii* (**LLee**) was reciprocally crossed with *Rexii*$^{\mathbf{Ki}}$ (**llee**), and the hybrids (**Llee**) then crossed reciprocally with **llEE**. Flying bombs interfered with the making of these crosses, but the results from the surviving material are given in Table 13. On the scale of these crossings there is no evidence that significant differences occur between the rates of growth of **L** and **E** pollen tubes, or between the viability of **L** and **E** egg-cells.

(2) *General.* It will be seen from Tables 7 to 10 that, in general, the segregation of the normal and sublethal characters deviates somewhat from 1 : 1. Where the heterozygote is used as female, this deviation must clearly be attributed to disturbed viability. But when the heterozygote is used as male there is often a significant excess of sublethals as compared with the results from the female, and in some male test-crosses this excess is very large. An excess of normals is rarely observed, though in some cases the reciprocal test-crosses do not appear to differ.

This can be attributed to specific isolation genes acting in the style in such a way as to discriminate in favour of the growth of pollen whose general genotype is most like that of the style itself. The F_1's of the species crosses are test-crossed to plants more nearly related to the parent from which they derived their sublethal genes than to the species which was their other parent. The parent contributing **L** to the F_1 was *S. Rexii*, and that contributing **E** was a garden form derived, like all garden forms, originally from hybridization of *S. Rexii* with *S. Dunnii*. Thus such specific isolation genes in *Rexii* would favour pollen-carrying genes for the garden strain more than that carrying genes for other species, and the garden strain would similarly favour pollen-carrying *Rexii* genes.

In the test-crosses with F_1 as female, such isolation genes will not be operative because the pollen is all *Rexii* or all garden strain. Pollen-carrying **L** or **E** may be less successful because of the action of **L** or **E**, or genes linked with them, but this is not a case of the action of specific isolators. These isolation genes can only be detected by comparison of the segregation in male and female test-crosses. The former should show more sublethals than the latter, since **L** and **E** are markers of the *Rexii* and garden strain chromosomes. This is, of course, what is observed. It has also been observed in a *Petunia* species-hybrid by Mather (1943), who discusses the problem in more detail.

DISCUSSION

Hybrid inviability, caused by genes of the type dealt with in this paper, has been reported in the literature a number of times, and relates to both interspecific and intraspecific inviability. The interspecific cases include *Crepis* (Hollingshead, 1932), *Nicotiana*

(Kostoff, 1936), *Epilobium* (Lehmann, 1939), and *Hutchinsia* (Melchers, 1939); also *Triticum* × *Aegilops* (Sears, 1940). The intraspecific cases include *Gossypium* (Hutchinson, 1932; Silow, 1941), *Hordeum* (Wiebe, 1934), and *Triticum* (Caldwell & Compton, 1943; Heyne *et al.* 1943). *Streptocarpus* belongs to the intraspecific category, since the sublethal genes have been found only in *S. Rexii.*

Dobzhansky (1937) has suggested that complementary genes such as **L** and **E**, once the respective populations carrying them had become geographically isolated, could bring about speciation, by preventing effective (i.e. capable of bringing about gene transference) intercrossing should the populations again come together. He points out that a basic postulate of this assumption is that the development of physiological isolation is preceded by geographical isolation of parts of the population. Let us see, therefore, what light is thrown on this point by *Streptocarpus.*

S. Rexii has the widest distribution of all the species of *Streptocarpus*, reaching from the extreme south of Cape Province to as far north as the Transvaal, the density of the population diminishing from south to north. The *Rexii* plant carrying the **L** gene came from Knysna. The plants carrying the **E** gene came from Balfour, 230 miles in a north-easterly direction from Knysna. These **E** plants, though clearly belonging to the species *Rexii*, are distinct from the Knysna plant in various ways, the differences in general being quantitative in character. One qualitative difference, however, in the **E** plants is their white flowers; they are recessive for the gene which controls anthocyanin production (Lawrence, Scott-Moncrieff & Sturgess, 1939). One other white-flowered form of *Rexii* is known to me, and this comes from Stutterheim, 47 miles east of Balfour. It, too, is distinct from the Knysna and Balfour plants, and carries neither **L** nor **E**.

Thus, although more evidence is required about the distribution of the variants of *Rexii* in South Africa, we find that the genes **L** and **E** come from widely separated populations, and appear to have arisen in geographically isolated groups, which are recognizably different. This situation can be stated another way: the genes **L** and **E**, like the white-flower character, seem to have arisen sporadically in different parts of the population of *S. Rexii.*

The first requirement of Dobzhansky's postulate is therefore satisfied; the **L** and **E** populations are geographically separated. We have now to inquire whether, if these populations ever meet, the **L** and **E** genes could act as an isolating mechanism leading to speciation.

Controlled cross-pollinations between the various forms of *Rexii* grown at Merton have shown each of them to be fully and reciprocally fertile with the others, and the F_1's also are fully fertile. There is, therefore, no inherent reason why the **L** and **E** populations, once they were brought together, should not intercross successfully. As a matter of fact, mixed pollinations made between all combinations of certain forms of *Rexii* and other rosulate species of *Streptocarpus* (Lawrence, unpublished) have shown a marked tendency for foreign pollen to compete successfully with self-pollen. That is to say, incipient self-incompatibility characterizes the rosulate species, including *Rexii* and its forms, and intercrossing is favoured, the hybrids showing considerable vigour. Thus, if no other factor intervened, intercrossing of the **L** and **E** populations would be encouraged.

Superficially, therefore, it seems that intercrossing of the **L** and **E** populations could occur and would even be encouraged, and the **LE** genes would then act as an isolating mechanism in speciation. Actually, it is extremely unlikely that this would be the case.

If **L** and **E** are to spread so as to become fixed, each in its own population, they must have an initial selective advantage. But Mather & Edwardes (1943) have pointed out that genes like **L** and **E**, which cause hybrid incapacity when they are brought together, are deleterious from the point of view of the immediate parents, and will therefore tend to be eliminated if crossing occurs at all. Hybrid incapacity, in other words, cannot of itself lead to speciation. On the contrary, it is always the consequence of a bar to crossing and cannot initiate speciation, though it may reinforce a bar to crossing.

Now a very common bar to crossing is the failure of foreign pollen to compete successfully with a plant's own pollen as it grows down the style. Muller (1942) has shown that genes producing a bar to crossing can have a selective advantage, in preventing the wastage of good eggs by fertilization with sperm (or pollen) which would lead to their developing into incapacitated hybrids. Such a bar to crossing would lead to the breaking up of the population into two groups, and this genetical isolation can thus lead to speciation.

We have seen that, in general, cross-pollination appears to be favoured in *Streptocarpus*. We might, nevertheless, ask whether the **L** and **E** populations arose some time in the past as the result of a bar to crossing involving the rates of growth of the pollens carrying **L** and **E**. If there ever was a difference in the growth rates of **L** and **E** pollen, it was not disclosed by the tests described earlier in this account.

The evidence from *Streptocarpus*, therefore, must be regarded as negative in so far as the role of genes **L** and **E** in speciation is concerned.

SUMMARY

When the two dominant genes **L** and **E** occur together in *Streptocarpus Rexii*, they give rise to abnormal foliar growth, though flowers and seeds, when produced, are normal. These genes have not been found in twenty other species of the genus. Both occur in wild populations, and **E** has also been found in the garden hybrids derived from species crosses within *Rexii* some 50 years ago.

Mutations and chimaeras are described One of the latter gave rise to tetraploids, the first to be recorded in garden *Streptocarpus*.

It is considered doubtful whether these genes can play a part in speciation.

REFERENCES

CALDWELL, R. M. & COMPTON, L. E. (1943). Complementary lethal genes in wheat causing a progressive lethal necrosis in seedlings. *J. Hered.* **34**, 67–70.

DOBZHANSKY, TH. (1937). *Genetics and the Origin of Species.* New York: Columbia University Press.

HEYNE, E. G., WIEBE, G. A. & PAINTER, R. H. (1943). Complementary genes in wheat. *J. Hered.* **34**, 243–5.

HOLLINGSHEAD, L. (1932). A lethal factor in *Crepis* effective only in an interspecific hybrid. *Genetics*, **15**, 114–40.

HUTCHINSON, J. B. (1932). Crumpled, a new dominant in Asiatic cottons produced by complementary factors. *J. Genet.* **25**, 281–91.

KOSTOFF, D. (1936). Polyploid hybrids *Nicotiana motica* var. *texana* L. × *Nicotiana glauca* Grah. *Bull. Appl. Bot., Genet., Plant Breed.*, ser. 2, no. 9, 153–62.

LAWRENCE, W. J. C., SCOTT-MONCRIEFF, R. & STURGESS, V. C. (1939). Studies on *Streptocarpus*. I. Genetics and chemistry of flower colour in the garden strains. *J. Genet.* **38**, 299–306.

LEHMANN, E. (1939). Zur Genetik der Entwicklung in der Gattung *Epilobium*. II. *Mitt. Jb. wiss. Bot.* **88**, 284–343.

MATHER, K. (1943). Specific differences in *Petunia*. I. Incompatibility. *J. Genet.* **45**, 215–35.

MATHER, K. & EDWARDES, P. M. J. (1943). Specific differences in *Petunia*. III. Flower colour and genetic isolation. *J. Genet.* **45**, 243–60.

MELCHERS, G. (1939). Genetik und Evolution. *Z. indukt. Abstamm.- u. VererbLehre*, **76**, 229–59.

MULLER, H. J. (1942). Isolating mechanisms, evolution and temperature. *Biol. Symp.* **6**, 71–125.

SEARS, E. R. (1940). Monofactorially conditioned inviability of an intergeneric hybrid in the Triticinae. *Genetics*, **25**, 133.

SILOW, R. A. (1941). The comparative genetics of *Gossypium anomalum* and the cultivated cottons. *J. Genet.* **42**, 259–358.

WIEBE, G. A. (1934). Complementary factors in barley giving a lethal progeny. *J. Hered.* **25**, 273–4.

EXPLANATION OF PLATES 1–4

PLATE 1

Fig. 1. Normal and sublethal seedlings, about 10 weeks old, from the cross **llEe** × **LLee**.

Fig. 2. Normal and sublethal seedlings, 10 weeks old, from the cross **LlEe** × **LLee**. × 2. The second cotyledon can still be seen in the normal seedling.

Fig. 3. Sublethal seedlings, some with narrow and some with broad cotyledons and leaves.

PLATE 2

Fig. 1. Showing the variation obtained in some families, ranging from extreme sublethal (1) to fully normal (5, 6). All seedlings 12 weeks old. The lethals are all **LlEe**.

Fig. 2. A narrow-cotyledoned sublethal, 5 months old. × 2.

Fig. 3. Three types of sublethals (all **LlEe**) from the same family: left, a broad-leaved plant, with a few narrow leaves; middle, a narrow-leaved plant, bearing a normal flower bud; right, a weak broad-leaved plant. All 20 months old.

PLATE 3

Fig. 1. *Streptocarpus Rexii* (type), **LLee**.

Fig. 2. Two of the test plants **llEE**.

PLATE 4

Fig. 1. A sublethal plant in which somatic mutation of **L** to **l** has given rise to two normal 'rosettes'.

Fig. 2. A cotyledon which, starting as sublethal, by somatic mutation gave rise to normal tissues at the base (cf. Pl. 2, fig. 2).

Fig. 3. Normal and sublethal leaves in a probable chimaera, which gave anomalous breeding results.

Fig 1.

Fig 2

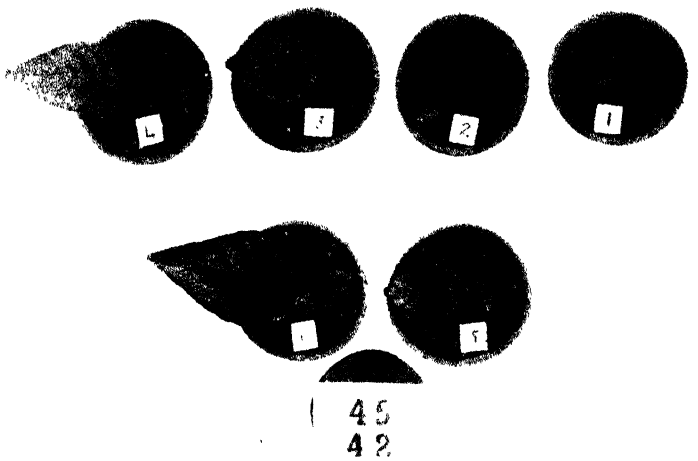

Fig. 1.

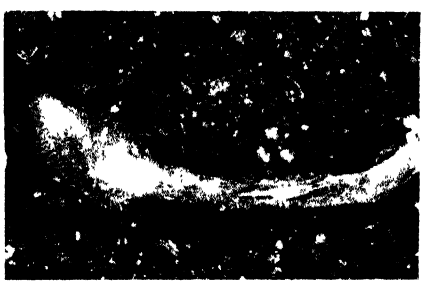

Fig. 2

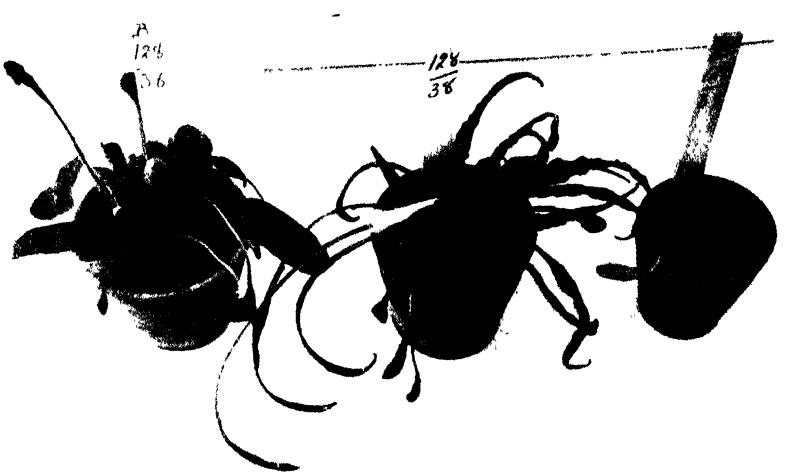

Fig. 3

Fig. 1.

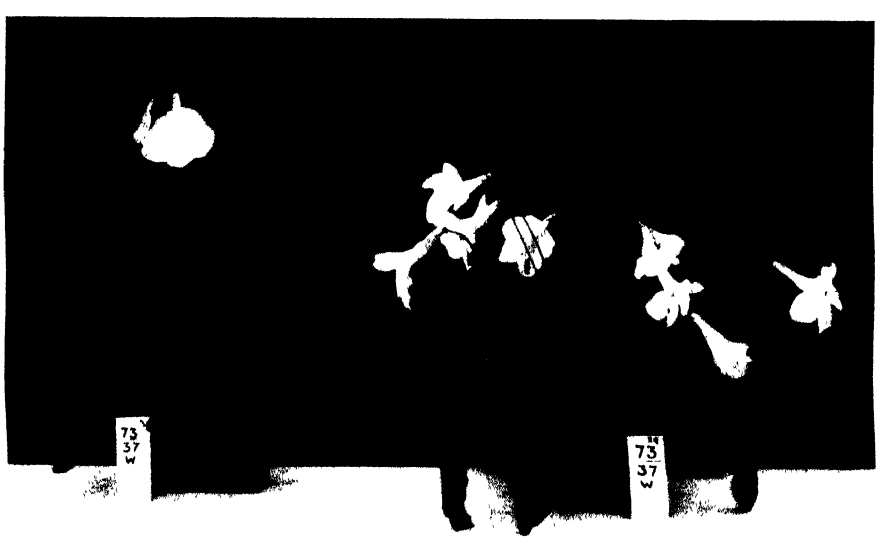

Fig. 2.

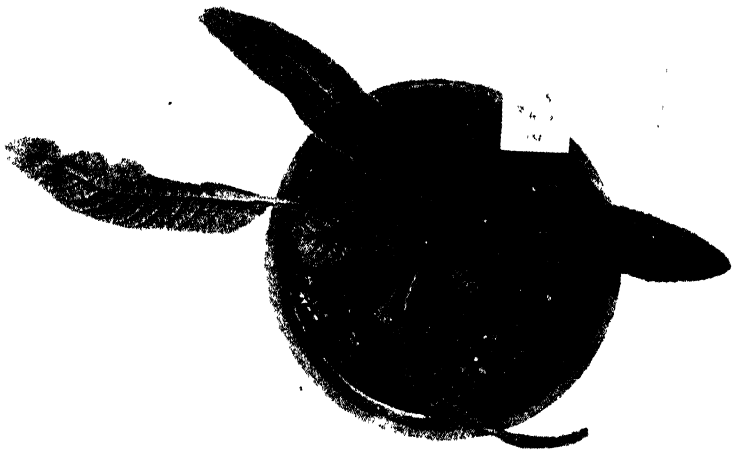

Fig. 1.

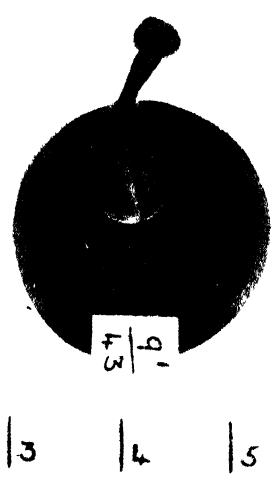

Fig. 2.

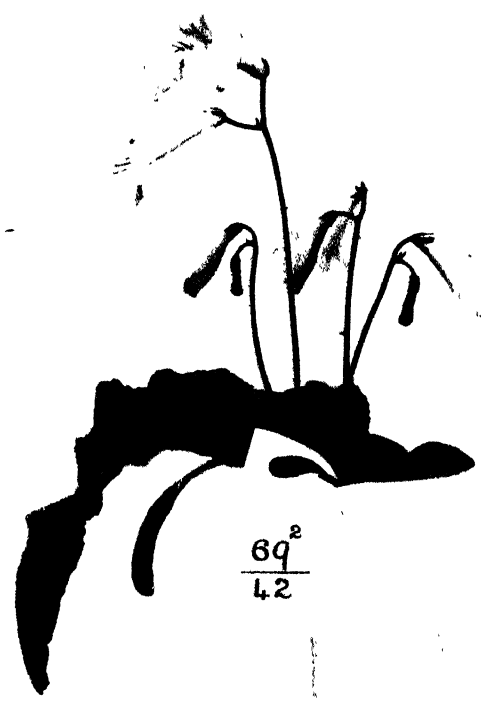

Fig. 3.

THE *P*-LOCUS POSITION EFFECT IN *OENOTHERA*

By D. G. CATCHESIDE, *Botany School, Cambridge*

(With Two Text-figures)

1. Introduction

The behaviour of a gene, when in the presence of a given set of other genes and under given environmental conditions, may depend upon its position in the chromosomes. Thus, gene *B* normally set between genes *A* and *C*, i.e. as *A B C D* ..., may have a different phenotypic expression when abnormally set through a structural change between genes *A* and *X*, i.e. as *A B X* .... This is the genetic phenomenon known as 'position effect'. Its proof, to the exclusion of mutation or other possible explanations, depends upon showing that the gene itself is identical when in the two positions. This may be demonstrated by transferring the abnormally acting gene to a normal position or by inserting a known normal gene into the abnormal position. Both these removals may be accomplished by crossing-over in suitable heterozygotes.

In *Drosophila melanogaster* numerous cases of position effect have been found, most of them involving changes in the dominance of normal wild-type genes. Two, or possibly three, types of effect may be distinguished, at least superficially. First, an interchange or other structural rearrangement with one break close to a particular locus results in the structural heterozygote showing a change (usually a loss) of dominance of the wild-type allelomorph whether the latter is located on the rearranged chromosome (*R*) or not. This happens, for instance, when *ci* (*cubitus interruptus* in chromosome IV) or its wild-type allelomorph ci^+ is removed to a position adjacent to euchromatin of any other chromosome (Dubinin & Sidorov, 1934 *a*, *b*; Stern & Heidenthal, 1944); it is sometimes referred to as the Dubinin-effect. In such cases, the structural heterozygote that is also ci/ci^+ shows the *cubitus interruptus* phenotype, but the structural homozygote does not. $R(ci)/+$ genotypes produce *ci* phenotypes in varying degree in contrast to $ci/+$ which is nearly normal. $R(ci)/ci$ genotypes produce more extreme *ci* phenotypes than ci/ci, the degree of deviation from normality being greatest for those $R(ci)$ which cause greatest abnormality with $+$. Also, $R(ci^+)/ci$ give strong *ci* phenotypes, but $R(ci^+)/R'(ci)$ show a shift towards a normal phenotype. Remarkably, those $R'(ci)$ which cause the most extreme *ci* phenotypes with ci^+ and *ci* are most effective in causing a shift to a normal phenotype with $R(ci^+)$.

In this type it is not clear whether the position effect is restricted in occurrence to those cases in which the affected locus *B*, originally set as ... *A B C D* ..., has a different locus actually adjacent to it, as in ... *A B X* ..., or whether it may also occur when the locus has a new neighbour not actually adjacent to it, as in

... *A B C X* ... or ... *A B C D X* ....

At any rate, such properties are shown by the second type, in which a wild-type gene in the neighbourhood of a breakage point of a structural change may, in a heterozygote carrying a recessive mutant allelomorph on the normal chromosome, be exhibited as a mottled or variegated phenotype made up of apparently normal tissue together with apparently mutant tissue. Where the structural homozygote is viable, it also shows the

variegation. Such behaviour has, for example, been shown (Schultz, 1936; Dubinin, 1936; Saccharov, 1936) to be associated with breaks in the neighbourhood of the w^+ (white) locus of the X-chromosome. When the break is just to the left of the w^+ locus, variegation may be exhibited not only for w^+ itself but also for the neighbouring loci rst^+ (roughest), fa^+ (facet), dm^+ (diminutive) and in some cases ec^+ (echinus) with decreasing intensity as the distance of the gene from the breakage locus increases. In variegated white, and likewise in other examples, the effect is commoner, more marked and extends to more genes, i.e. to a greater length of chromosome, if the region affected is translocated to the neighbourhood of heterochromatin, rather than euchromatin (Demerec, 1940). The variegation is partially or completely suppressed by growth at high temperature (Gowen & Gay, 1933*b*) or by the addition of one or more Y-chromosomes (Gowen & Gay, 1933*a*).

The third type of position effect in *Drosophila* is characterized by the production of a change that is semi-dominant to the normal wild type as, for example, in the duplications that are responsible for Bar eye (Bridges, 1936) and Hairy wing (Demerec & Hoover, 1939). We are here considering the comparison of B and $+$ rather than the position effect shown by the unlike phenotypes of $BB/+$ and B/B. It is possible that the distinction between this type and the first is more apparent than real. In the case of Bar, Sutton (1943) has shown by comparison with a Bar deficiency that the Bar locus in its normal position has no effect on the phenotype of the fly. The Bar effect is apparently produced by interaction of the Bar locus when in contact with certain other specific loci.

Lastly, it should be mentioned that in *Drosophila* structural changes when homozygous are often less viable or even lethal compared with normals, though whether such lethal effects are properly accounted for as position effects is doubtful (Lea & Catcheside, 1945).

There is no clear evidence for the occurrence of position effects in any other organism except *Oenothera* (Catcheside, 1939). Various cases have been reported where viability or fertility changes are associated with structural changes in the chromosomes. Thus Savchenko (1935) found an interchange in the vetch (*Vicia sativa*) that was less fertile as a homozygote than as a heterozygote. In maize, interchange gametes (or rather gametophytes) seem as viable as normal ones. Thus, when an interchange heterozygote is used as a pollen parent, equal numbers of interchange and of normal pollen tubes effect fertilization. Stadler (1941) showed this to be true of about fifty different interchanges in maize. This is in striking contrast to the behaviour in *Oenothera blandina* where, of ten X-ray induced interchanges, five showed reduced male gametophyte viability (Catcheside, 1935). Similar effects are shown in *Datura* (Blakeslee & Bergner, 1940).

In maize, Jones (1939, 1944) has found various colour and growth changes in the endosperm to be associated with the relocation of chromosome parts. Brink (1932) found plants homozygous for a particular interchange to be slightly earlier than normal ones. Roberts (1942), in an extensive replicated test of a number of maize interchanges, has found similar effects on vigour and earliness in both homozygous and heterozygous interchange plants.

In none of these cases, however, can it be said that the production of gene mutation concurrent with the structural change has been excluded. In one case in *Oenothera blandina* it was possible to give proof of position effect (Catcheside, 1939) though the proof was weak. This weakness lay in the possibility that the single critical plant could itself have been a mutant or even an interloper. These possibilities are removed by the experiments reported below.

2. THE P^s POSITION EFFECT IN *OENOTHERA BLANDINA*

The P locus lies in arm 3 of chromosome 3.4 of *O. blandina*. A position effect, acting on the P locus, was found (Catcheside, 1939) in an X-ray induced interchange between chromosomes 3.4 and 11.12 in which the new chromosomes were 3.11 and 4.12 (Catcheside, 1940). Plants heterozygous for the interchange are characterized by narrower leaves than normal *blandina* and by a peculiar greyish green leaf colour. The interchange set of chromosomes is conveniently referred to as *blandina*-A and by the symbol A, the normal set as ^{h}*blandina* and by the symbol +. The position effect was exhibited in relation to P^s, a P allelomorph in which the sepals of the flower buds show broad uniformly red stripes separated by narrow green or yellow-green ones (Fig. 1*a*). In heterozygous plants that

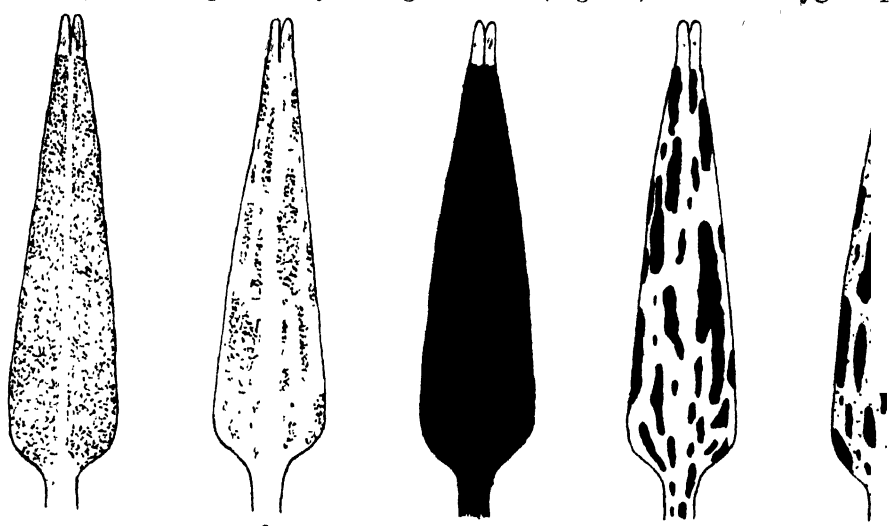

Fig. 1. Diagrams of pigment distribution in buds of various genotypes. The black areas represent deep red, the dotted areas light red and the white areas green tissue. (*a*) P^s P^s *blandina*; (*b*) P^s ^{h}*blandina*/P^s *blandina*-A; (*c*) P^r P^r *blandina* or P^r P^s *blandina* or P^r ^{h}*blandina*/P^s *blandina*-A; (*d*) P^s ^{h}*blandina*/P^r *blandina*-A.

are P^s ^{h}*blandina*/P^s *blandina*-A, the sepals show more or less numerous streaks of green so that the red pigmentation is broken up into patches of varying size (Fig. 1*b*). The green pigmented areas show a colour which is characteristic of the two lower allelomorphs at the P locus, namely, P and p. P^s is incompletely dominant over P and p, the buds of P^s P and P^s p plants being green merely flushed with red. The position-effect sepals show a mosaic of P^s P^s and apparently P^s P or P^s p tissue with sharp boundaries between the areas. The sepals are variegated in a manner analogous to the eyes in *Drosophila melanogaster* stocks that have a translocation of the w^+ locus to a position close to heterochromatin. In the *Oenothera* case, there is no direct evidence that a translocation of P^s to the neighbourhood of heterochromatin is involved, but the analogous genetic behaviour described below is suggestive of this. The proof that the variegation was a position effect consisted in transferring the affected P^s gene from chromosome 3.11 by crossing-over into a normal 3.4 chromosome. When this was done, the variegation disappeared and the P^s gene was restored to its full activity. Thus there was no evidence that any mutation had been induced in it at the same time that the interchange was produced.

The original proof (Catcheside, 1939) was founded upon a single critical plant. It was, therefore, important to secure confirmatory evidence, particularly by inserting another

P allelomorph, namely, P^r (rubricalyx), which produces uniformly deep red sepals, into the interchange chromosome. The present paper describes the results, which fully confirm the position-effect hypothesis. Briefly, P^s and P^r show variegation when in the 3.11 chromosome, and the variegation extends to the neighbouring *S* locus. It is convenient to record the phenotypes by the symbols P^{se}, P^{re} and S^e in the cases of plants showing the variegation. In addition, various unexpected and complicating phenomena were discovered. These are capable of explanation as duplications (Fig. 1*e*) or deficiencies respectively of the *P-S* region; discussion of them is deferred to a later paper, though the identifications of the plants are inserted into the tables of the present paper where desirable.

3. Substitution of P^r for P^s in chromosome 3.11

The technique of substitution was as follows: P^s ^{h}*blandina*/P^s *blandina*-A plants were crossed with P^r P^r *blandina* plants and the P^r ^{h}*blandina*/P^s *blandina*-A plants picked out amongst the progeny. This could be done readily at the seedling stage using the character of the narrow leaves and their peculiar green colour. These seedlings also show the presence of red pigment in the epidermis of the underside of the mid-veins of the leaves, a character determined by P^r. The flower buds of these plants were deep red (Fig. 1*c*) without any sign of variegation. These doubly heterozygous plants were then back-crossed to P^s P^s *blandina*. In 1940, 717 plants in five families of this backcross were grown; the distribution of these plants amongst the various types is summarized in Table 1.

Table 1. *Progenies of* P^s blandina-*A*/*P* ^{h}blandina × P^s P^s blandina

Genotype	Family 68/40	69/40	70/40	71/40	72/40	Totals
P^s *blandina*-A/P^s ^{h}*blandina*	20	73	47	60	122	322
P^r ^{h}*blandina*/P^s ^{h}*blandina*	35	97	54	65	119	370
P^s ^{h}*blandina*/P^s ^{h}*blandina*	.	2	2	1	.	5
P^r *blandina*-A/P^s ^{h}*blandina*	.	1	.	1	.	2
P^r P^s *blandina*-A/P^s ^{h}*blandina* (duplication)	.	.	.	.	1	1
P^s P^r *blandina*-A/P^s ^{h}*blandina* (duplication)	.	1	.	1	.	2
– ^{h}*blandina*/P^s ^{h}*blandina* (deficiency)	.	2	1	1	2	6
– *blandina*-A/P^s ^{h}*blandina* (deficiency)	.	1	2	1	1	5
Trisomics	1	1	.	1	1	4
Totals	56	178	106	131	246	717

The transfer of the affected P^s gene from the 3.11 chromosome to a normal position in a 3.4 chromosome restores its normal behaviour. This was found in five plants which had buds indistinguishable from regular P^s P^s *blandina* plants (Fig. 1*a*).

The P^r gene was transferred into the 3.11 chromosome in five plants, but in the case of three of them the transference involved the establishment of a duplication. Discussion of these three plants is deferred. In the other two plants the sepals showed deep red pigmented areas interrupted by pure green areas forming a green background (Fig. 1*d*). Thus P^r in chromosome 3.11 shows a position effect similar to that of P^s. Restoring this affected P^r gene to a normal 3.4 chromosome, as was done in another similar experiment, restores the normal behaviour of the gene.

All the observed transfers of either P^s or P^r from 3.4 to 3.11 or vice versa are enumerated in Table 2. In each case, insertion of the gene into 3.11 led to the characteristic position effect, while the removal of the position-affected gene to 3.4 always resulted in the

restoration of its normal behaviour. The observation of fifty-eight critical transfers removes all possible doubt that we are dealing with a genuine position effect. No matter what phenotypic manifestations may follow upon placing P^s or P^r in the interchange chromosome, the P-locus genes are themselves unchanged in their structure, as is shown by their normal behaviour when replaced in a chromosome of normal structure.

Table 2. *Transfers of P^s, P^r and S between chromosomes* 3.4 *and* 3.11

Transfer		Gene		
From	To	P^s	P^r	S
3·4	3·11	17	7	4
3·11	3·4	17	17	71

4. Locus of the interchange break in chromosome 3.11

No detailed cytological localization is possible in *Oenothera*, the pachytene stage being unsuitable for analysis. The experiments designed to demonstrate the position effect (Table 1) showed that the interchange locus must be genetically close to the P locus. There were seven cross-overs amongst 699 plants classified, that is, omitting the duplication and deficiency plants and the four trisomics whose exact constitution was in doubt. Thus there is approximately 1 % crossing-over between P and the interchange break locus.

It is important to determine whether the interchange locus is to the left or right of P, i.e. distal or proximal with respect to the centromere. It is known that S (S = yellow petals; s = sulphur-coloured petals) is also carried on chromosome arm 3 (Emerson & Sturtevant, 1932; Catcheside, 1940) and that the order is S-P-centromere, with about 8 % crossing-over between S and P. *Blandina* plants homozygous for s and P^s were constructed, being extracted from $S\ P^s/s\ P^r$ by selfing. Several families were then grown (Table 3) from matings of the type $A\ P^r\ s/+P^s\ S \times +P^s\ s$, the type $A\ P^s\ S/+P^r\ s \times +P^s\ s$ and the type $A\ P^r\ S/+P^s\ s \times +P^s\ s$. It was found in making up the heterozygotes that, whereas $A\ s/+S$ had normal yellow petals, $A\ S/+s$ had petals that were a mosaic of yellow- and sulphur-coloured patches. Some of the sulphur patches were large, occupying as much as half a petal or more, but most were quite small. The general effect of the yellow and sulphur mosaic is to give the petals a colour which, seen at a distance, is intermediate between yellow and sulphur. At close range, the patchwork is quite obvious. Moreover, the boundaries between the yellow and sulphur areas are perfectly sharp, there being no appearance of any intergradation between the two colours. Thus S also shows a variegation like that of P^s or P^r and under similar conditions. In both cases the variegation is more extensive earlier in the season, that is, in the buds first produced. Later in the season, in the later produced buds, the variegation becomes much less extensive, and in the case of S variegation is often not at all obvious in the petals of the flowers produced in the cooler weather in September. These are purely physiologically determined variations in expression, dependent upon senescence and environment; there is no difference between progeny grown from early and late flowers of the same plant.

The details of the backcross test progenies are given in Table 3 and summarized in Table 4. The test shows that the P locus is closer to the interchange locus than is the S locus, and that the order of these three points is interchange-P-S. Nearly all cross-overs in the interchange-P interval are also cross-overs between the interchange locus and S; there are only two exceptions (double cross-overs) amongst nineteen cross-overs in the interchange-P interval. There is 1·7 % recombination in the interchange-P interval and

8·5% recombination in the *P-S* interval. Thus 0·15% of double cross-overs could have been expected where 0·18% were found.

The assignment of precise linkage values to the region concerned is complicated, especially in the interchange heterozygote by the occurrence of unequal crossing-over leading to duplications and deficiencies. This is rather frequent in comparison with regular crossing-over. In the case of the interchange-*S* region, the value is distorted by the different gametic viabilities of *S*- and of *s*-carrying gametes. However, the data hardly warrant more elaborate treatment.

Table 3. *Three-point test-crosses of S P and interchange break locus in chromosome* 3.4

Family	Genotypes of parents	Phenotypes of offspring: *Blandina* $P^s S$	*Blandina* $P^s s$	*Blandina* $P^r S$	*Blandina* $P^r s$	*Blandina*-A. ^{h}*blandina* $P^{se} S^e$	$P^{se} s$	$P^{re} S^e$	$P^{re} s$	Exceptions	Total plants
11/43	$\frac{+P^s S}{A P^r s} \times \frac{+P^s s}{+P^s s}$	43	1	.	2	.	.	4	19	2 *Dp*	72
								(1)			
12/43	$\frac{+P^r s}{A P^s S} \times \frac{+P^s s}{+P^s s}$	4	.	19	75	112	6	1	1	2 *Dp*	220
13/43	$\frac{+P^r s}{A P^s S} \times \frac{+P^s s}{+P^s s}$	4	.	7	57	87	6	1	.	5 *Dp*	167
14/43	$\frac{+P^r s}{A P^s S} \times \frac{+P^s s}{+P^s s}$	.	.	2	46	108	1	.	.	2 *Dp*	159
15/43	$\frac{+P^r s}{A P^s S} \times \frac{+P^s s}{+P^s s}$	2	.	6	60	78	5	.	.	2 *Dp*	155
						(2)					
16/43	$\frac{+P^r s}{A P^s S} \times \frac{+P^s s}{+P^s s}$	.	.	11	49	95	6	.	1	.	169
						(7)					
19/44	$\frac{+P^s s}{A P^r S} \times \frac{+P^s s}{+P^s s}$	2	33	.	.	.	2	25	5	3 *Dp*; 1 *Df*	72
								(1)			
33/44	$\frac{+P^s s}{A P^r S} \times \frac{+P^s s}{+P^s s}$	6	64	.	.	.	1	50	5	3 *Dp*; 3 *Df*	166
		(14)				(1)		(19)			

Notes. Numbers in brackets refer to plants that failed to flower, so petal colour could not be scored. In column listing exceptions, *Dp* are duplications and *Df* are deficiencies.

Table 4. *Summary of linkage data for S-P-interchange break*

Family	Non-cross-overs	Single cross-overs: Region 1	Single cross-overs: Region 2	Double cross-overs: Regions 1 and 2	Total plants
11/43	62	2	5	.	69
12/43	187	5	25	1	218
13/43	144	4	13	1	162
14/43	154	.	3	.	157
15/43	138	2	11	.	151
16/43	144	1	17	.	162
19/44	58	2	7	.	67
33/44	114	1	11	.	126
Total	1001	17	92	2	1112

Note. Duplications, deficiencies, and incompletely scored plants omitted from the reckoning.

The position effect thus extends over a remarkably long genetic distance, about 10 units, much longer than in any of the *Drosophila* cases. It is possible that the cytological distance is small, but there seems to be no likelihood of obtaining direct evidence by observation of the chromosomes.

The data show that the interchange is one of the kinds shown diagrammatically in Fig. 2. There is no direct method available to decide between these alternatives, but on

general grounds (*b*) is the more likely, namely, that the break in 3.4 occurs between P^s and the centromere. If it becomes possible to analyse the pachytene stage of meiosis, the point could be settled. The nature of the duplications obtained shows in an indirect way that this must be the structure, otherwise the duplications would have dicentric chromosomes.

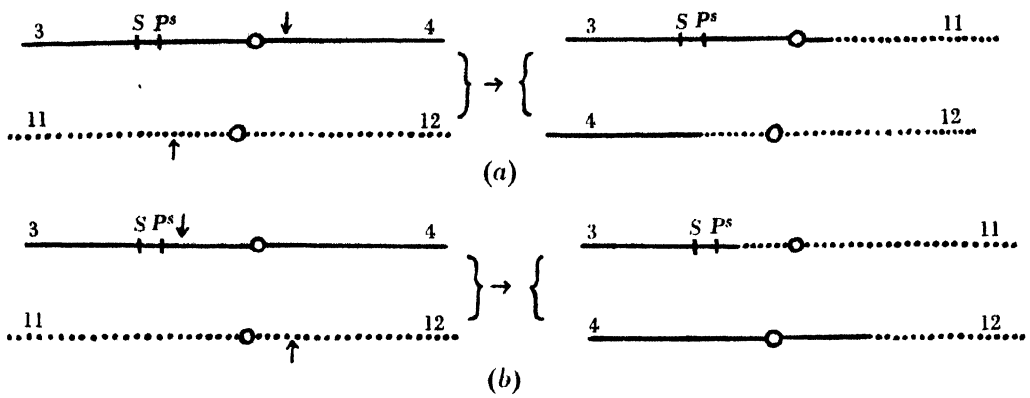

Fig. 2. Diagrams of the possible origins and structures of the 3.11 + 4.12 interchange in *Oenothera blandina*; (*b*) is the more probable on general grounds.

5. EGG AND POLLEN TRANSMISSION OF INTERCHANGE GAMETES

In general, gametophytes carrying the interchange are somewhat less viable in competition with normal gametophytes both as embryo sacs and as pollen tubes that function in fertilization. The available data are given in Table 5. A χ^2 test shows that the forty-eight families available to estimate the relative embryo sac production of normal and interchange megaspores are highly heterogeneous ($\chi^2 = 111{\cdot}6829$ for 47 degrees of freedom). The

Table 5. *Numbers of functional normal and interchange gametophytes yielded by various genotypes*

(A) + P^s S/A P^s S		(B) + P^s S/A P^r S		(C) + P^r S/A P^s S		(D) + P^s s/A P^s S	
+	A	+	A	+	A	+	A
Embryo sacs		Embryo sacs		Embryo sacs		Embryo sacs	
29	33	84	78	46	50	3	3
64	63	113	76	28	21		
55	52	138	125	36	20		
30	28	66	33	100	78		
78	72	57	50	56	50		
8	10	137	132	67	64		
31	33	63	61	120	126		
73	39	42	29	47	66		
94	69	39	33	Total 500	475		
Total 462	399	35	37				
		68	50				
Pollen grains		75	80				
82	70	Total 917	784				
56	47	Pollen grains					
10	14	15	17				
41	28						
83	77						
48	24						
5	3						
Total 325	263						

(E) + P^s S/A P^r s		(F) + P^s s/A P^r S		(G) + P^r s/A P^s S		(H) + P^s s/A P^s s	
Embryo sacs		Embryo sacs		Embryo sacs		Embryo sacs	
46	23	36	36	41	40	11	15
		87	79	44	43	8	10
		Total 123	115	99	119	Total 19	25
		Pollen grains		68	94		
		29	41	48	109		
				68	85		
				60	109		
				Total 428	599		

(I) + P^s s/A P^r s	
Embryo sacs	
109	86
18	12
Total 127	98

nine genotypes, into which the data may be grouped, show a χ^2 of 55·6487 for 8 degrees of freedom, indicating a high degree of heterogeneity as between genotypes. The χ^2 for the remaining 39 degrees of freedom is 56·0342, P lying between 0·08 and 0·09. Thus the heterogeneity may be ascribed entirely to effects of the different P- and S-locus compositions. The precise way in which P^s and P^r and S and s affect megaspore competition cannot be deduced satisfactorily from the present material. Only nine of the possible sixteen genotypes heterozygous for the interchange are available, and in several of these the data are scanty. It may be said, however, that P^r relative to P^s appears to cause a slightly depressive effect on megaspore viability, while s relative to S causes a more marked depression. No doubt these effects come about through slight Renner effects, but no direct evidence exists.

Amongst fertilizations effected by pollen also there is a small deficiency of those resulting from pollen tubes carrying the interchange.

6. Viability and appearance of interchange homozygotes

During the first generations after the origin of the interchange it was found that interchange homozygotes could not be reared beyond the seedling stage. The early leaves were small, thickened and distorted; and after a shorter or longer time the growing point of the stem went awry and no more growth occurred. The root system was also rather deficient. For most of the period of the experiments described above, the stocks were maintained by crossing to homozygous normals. Occasional selfed progenies were grown and the homozygotes were usually of the expected crippled type.

In one family grown in 1943 the interchange homozygotes seemed more vigorous than usual, and eight of them were successfully overwintered in a box in the cool greenhouse. In 1944 four plants flowered, the others having been lost through planting out at the beginning of a dry spell. This family had arisen from selfing a heterozygote that was P^r *blandina*-A/P^s ^{h}*blandina*. All the interchange homozygotes were homozygous for P^r. The four plants that flowered all showed P^r variegation though less extensive than in A $P^r/+P^s$ plants. The appearance of the sepals is such as would be expected if two P^r variegations were superposed. The plants were not examined for S variegation. The general habit of the plants was like that of the heterozygotes but more extreme, the plants being quite small.

The present case of position effect thus agrees with those cases of w^+ variegation in *Drosophila* which show in homozygotes if the latter are viable, rather than with *ci* position effects which are suppressed in homozygotes.

7. Discussion

The existence of the position-effect phenomenon in *Oenothera* has been clearly established by the above experiments. It remains to consider what bearing the observations may have on theories seeking to explain position effect. In the first place it is quite clear that the P^r, P^s and S genes are themselves unaltered in structure by their presence in the abnormal position. At least the genes recovered from the abnormal position show no abnormality, so that any change in the genes wrought while they are position affected must be temporary and at once reversible when they are removed from the local influence. This behaviour makes it difficult to accept the suggestion of Demerec & Slizynska (1937) that

position-effect variegations are frequent somatic mutations arising as a result of an induced mutability in the affected genes.

The region of the chromosome at the locus of the interchange appears to act as a modifier on various genes more or less closely linked to it, provided in the case of variegations that the genes are actually in the abnormal chromosome. This circumstance limits the possible mechanisms that must be explored. Thus anything closely analogous with mechanisms that would account for the interaction of genes in separate chromosomes in the same nucleus will not suffice.

If we seek for an explanation in terms of diffusion (Sturtevant, 1925) with localized interaction of gene products or competition for precursors, we must suppose that the capacity for diffusion is limited. This limitation is imposed by two circumstances. The diffusion may not spread in certain directions, for genes situated on a separate chromosome are unaffected. In *Drosophila*, particularly, in which there is regularly strong somatic pairing, the homologous parts of the chromosomes must be very close together in a great majority of somatic cells and yet show no interaction comparable to position effect. On the other hand, diffusion along the chromosome for some distance must be possible since genes situated at a considerable distance from the source of the effect are modified in their action.

In contrast to such kinetic hypotheses (Ephrussi & Sutton, 1944) are structural hypotheses (Muller, 1935; Dobzhansky, 1936). The latter postulate reversible modifications of the genes themselves or of their structural interrelations. For instance, it might be supposed that chemical bonds unite neighbouring genes into integrated, larger units, and that within such a unit, as within any large molecule, changes produced in one of its parts would affect the properties of the whole. However, the distances over which position effects may extend are so great in some cases, of the order of 100 gene diameters, that an explanation in terms of such steric effects seems inconceivable (Ephrussi & Sutton, 1944). Moreover, as these authors have also shown, the effects possible by ordinary diffusion at such great distances are so slight as to be completely negligible. A kinetic explanation is possible only if the spread of substances along the chromosome is substantially easier than radially outwards from the chromosome.

The explanation of position effect then must be sought in terms of some factor that will spread along or through a chromosome rather than outwards through the karyolymph. Unless diffusion from or to a gene is preferentially along the chromosome, we must conclude with Ephrussi & Sutton (1944) that the factor responsible is some change in the physical state of the chromosome itself. On analogy with the behaviour of myosin fibres, these authors suggest that the change is one involving the state of extension of the chromosome. In the Diptera, coincident with the occurrence of position effect, the chromosomes show an intimate association in somatic pairing. If, as seems likely, there is a rather precise alignment of homologous parts in somatic pairing, the presence of structural rearrangements in the heterozygous condition could lead through changes in the pairing to alterations in the state of extension of the chromosome in the vicinity of the breakage points. This hypothesis carries with it the corollary that the action should not be confined to the genes in the chromosome carrying the break, but should also appear in its homologue. This expectation is fulfilled in the *cubitus interruptus* position effects (Stern & Heidenthal, 1944) referred to earlier. The data of Dubinin & Sidorov (1934*a*, *b*) add the following corroborative facts, namely that any ci^+ translocations which show

position effects when heterozygous ($R(ci^+)/ci$) give a normal phenotype when homozygous or hemizygous, that is when pairing is complete or absent. Both would lack a local stress.

The cases of Bar and other similar contiguous duplications are also accounted for since the distortion is here intrachromosomal through synapsis of homologous parts of the two duplicated sections. We would therefore expect, as is indeed found, that homozygotes and hemizygotes as well as heterozygotes would have abnormal phenotypes.

The w^+ variegations, and similar ones of bw^+, present greater difficulties, since homozygotes and hemizygotes show the white mottled phenotypes. On analogy with the *ci* cases this is unexpected, for homozygotes show complete pairing and hemizygotes a lack of it. The case can be reconciled with theory on the assumption that the manifestation of the position effect depends upon the affected loci being adjacent to heterochromatin. The heterochromatin of *Drosophila melanogaster* shows non-homologous pairing, or more strictly the heterochromatin of different chromosomes is non-specific in its pairing perhaps because it is not much differentiated. Such non-specificity of pairing should extend to the parts of heterochromatin within one chromosome and such intrachromosomal pairing might well affect genes attached to the heterochromatin. This is the suggestion offered by Ephrussi & Sutton (1944), with the admission that it is tentative.

This explanation would also have to be applied to the *Oenothera* case, although there is no direct evidence that heterochromatin is involved. The evidence is the spreading of the effects along the chromosome coupled with the absence of somatic pairing rendering position effects analogous to the ci^+ and *B* ones improbable. There is no cytological evidence for the occurrence in *Oenothera* of somatic pairing, in fact the appearances are all against its existence in the plant. The possibility of somatic pairing must however be entertained. A number of chimerical plants have been found in *Oenothera*, in which the origin of one component from the other by intrachromosomal somatic crossing-over seems rather probable. The occurrence of somatic crossing-over implies somatic pairing, though it may be only of heterochromatic segments. These exceptional plants are referred to in a subsequent paper on duplication and deficiency in *Oenothera*.

The proposed explanation of the heterochromatin position effects of w^+ does not suggest why these particular position effects should spread further along the chromosome from the seat of action than in the cases of translocations of w^+ to the neighbourhood of euchromatin (Demerec, 1940). The effect could be accounted for if proximity to heterochromatin sometimes caused a persistence of nucleic acid on the genes during the resting stage of the nuclear cycle and if such a coating of nucleic acid inhibited the normal functioning of the gene. Such heterochromatinization of euchromatin bands translocated to the neighbourhood of the heterochromatin has been observed in *Drosophila* by Schultz (1941). This explanation is one of the factors accepted by Prokofyeva-Belgovskaya (1945), who interprets the effect as one of a reduction in the length of the metabolic stage of the affected chromosome region. She also draws attention to two other factors, namely, the effect due to the addition of extra heterochromatin (from the *Y*-chromosome) and an effect dependent upon whether the affected chromosome region was homo- or heterozygous in the parent. I have no sufficiently definite information upon which to judge whether a similar effect of heterozygosity exists in the *Oenothera* case.

A second peculiarity that is not at once accounted for by Ephrussi and Sutton's stress hypothesis, is the fact that the abnormal tissue is often in large patches as though all the cells in the patch were the descendants of one cell in which the abnormality had been

produced. This is very striking in the *Oenothera* case. Rarely, whole branches may appear green budded with the exception of an odd bud here and there which may show red patches on part or the whole of the bud. Even so, the progeny derived from such green buds on wholly green branches are entirely like those from a bud showing the usual variegation. The interchange heterozygotes have red and green variegated sepals, and cross-overs to a normal chromosome of the affected *P* allelomorph, in particular P^r, show it to have an unaltered structure. The observations suggest that the inhibition of the affected gene is by a mechanism that, having occurred by chance in a given cell, persists for a number of cell divisions, rather than by a mechanism that arises by chance in each cell generation and does not persist. No data are available to estimate the persistence time, but it can be stated that the period (in terms of division cycles) is greater at the beginning of the season than at the end; this may be a temperature effect, but one cannot be certain. We need to know whether the suggested intrachromosomal heterochromatin pairing does persist and whether the persistence is governed by external factors such as temperature. Presumably the effect might be secured by a semipermanence of the heterochromatinization earlier referred to. Another possibility that must be explored is the effect upon the differentiation and development of cytoplasmic genes capable of reproduction for a limited period subject to ultimate decay.

A third awkward fact is presented by Lewis (1945) in connexion with the Star-asteroid region of *Drosophila melanogaster* which is a reverse repeat, or reverse contiguous duplication, minute in size and located at the 21 E 1, 2 doublet in the left arm of chromosome II. The Star locus is on the first of these bands and the asteroid locus on the second. The phenotypes of *S ast*/+ + and *S*+/+ *ast* are widely different from one another. Here we have a case where on analogy with intrachromosomal duplications such as Bar, compared with +, we should, on the stress hypothesis, expect no phenotypic difference between the two genotypes. It is difficult to conceive how with the same stresses within each chromosome two genes could be modified differently according to whether they were together in the same chromosome or apart in separate homologues.

While the stress hypothesis will encompass the explanation of a surprising diversity of facts, one cannot avoid the conclusion that other mechanisms are also concerned.

SUMMARY

In *Oenothera blandina*, the genes P^s, P^r and *S* produce a variegated phenotype when they are present in the interchange chromosome 3.11. When transferred by crossing-over to a normal 3.4 chromosome, they produce normal phenotypes. The variegation is therefore a position effect.

The break is 1·7 units from the *P* locus and 8·5 units from the *S* locus, indicating a considerable spread of the position effect along the chromosome. The action is thought to depend on translocation of the *P* and *S* loci to the neighbourhood of heterochromatin.

Theories of the mechanism of position effect are considered, but the *Oenothera* case adds nothing new to the solution of the problem.

REFERENCES

BLAKESLEE, A. F. & BERGNER, D. (1940). *Yearb. Carneg. Instn*, **39**, 207–8.
BRIDGES, C. B. (1936). *Science*, **83**, 210–11.
BRINK, R. A. (1932). *Amer. Nat.* **66**, 444–51.
CATCHESIDE, D. G. (1935). *Genetica*, **19**, 134–42.
CATCHESIDE, D. G. (1939). *J. Genet.* **38**, 345–52.
CATCHESIDE, D. G. (1940). *Proc. Roy. Soc.* B, **128**, 509–35.
DEMEREC, M. (1940). *Genetics*, **25**, 618–27.
DEMEREC, M. & HOOVER, M. E. (1939). *Genetics*, **24**, 271–7.
DEMEREC, M. & SLIZYNSKA, H. (1937). *Genetics*, **22**, 641–9.
DOBZHANSKY, TH. (1936). *Biol. Rev.* **11**, 364–84.
DUBININ, N. P. (1936). *Biol. Zh.* **5**, 851–74.
DUBININ, N. P. & SIDOROV, B. N. (1934*a*). *Amer. Nat.* **68**, 377–81.
DUBININ, N. P. & SIDOROV, B. N. (1934*b*). *Biol. Zh.* **3**, 307–31.
EMERSON, S. H. & STURTEVANT, A. H. (1932). *Genetics*, **17**, 393–412.
EPHRUSSI, B. & SUTTON, E. (1944). *Proc. Nat. Acad. Sci., Wash.*, **30**, 183–97.
GOWEN, J. W. & GAY, E. H. (1933*a*). *Proc. Nat. Acad. Sci., Wash.*, **19**, 122–6.
GOWEN, J. W. & GAY, E. H (1933*b*). *Science*, **77**, 312.
JONES, D. F. (1939). *Genetics*, **24**, 100.
JONES, D. F. (1944). *Genetics*, **29**, 420–7.
LEA, D. E. & CATCHESIDE, D. G. (1945). *J. Genet.* **47**, 10–24.
LEWIS, E. B. (1945). *Genetics*, **30**, 137–66.
MULLER, H. J. (1935). *Proc. 15th Int. Physiol. Congr., Leningrad.*
PROKOFYEVA-BELGOVSKAYA, A. A. (1945). *C.R. Acad. Sci. U.R.S.S.* **47**, 360–2.
ROBERTS, L. M. (1942). *Genetics*, **27**, 584–603.
SACCHAROV, V. V. (1936). *Biol. Zh.* **5**, 293–302.
SAVCHENKO, P. F. (1935). *Bull. Appl. Bot. Genet. Plant Breed.* **8**, 59–79 (Russ.) (Eng. Sum. pp. 183–9).
SCHULTZ, J. (1936). *Proc. Nat. Acad. Sci., Wash.*, **22**, 27–33.
SCHULTZ, J. (1941). *Cold Spr. Harb. Symp. Quant. Biol.* **9**, 55–65.
STADLER, L. J. (1941). *Cold Spr. Harb. Symp. Quant. Biol.* **9**, 168–77.
STERN. C. & HEIDENTHAL, G. (1944). *Proc. Nat. Acad. Sci., Wash.*, **30**, 197–205.
STURTEVANT, A. H. (1925). *Genetics*, **10**, 117–47.
SUTTON, E. (1943). *Genetics*, **28**, 97–107.

THE GENETICS OF BLACKARM RESISTANCE

V. DWARF-BUNCHED AND ITS RELATIONSHIP TO $\mathbf{B_1}$

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(With Plate 5)

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Introduction

In the course of breeding work designed to produce blackarm-resistant American Upland types, crosses were made between Nye's Uganda B31 and 514. A number of bunched-up dwarfed plants appeared in the F_2 of this cross and, as dwarfing appeared to be associated with blackarm resistance, a knowledge of its genetics became desirable from the plant-breeding standpoint. In the present paper it is shown that one of the factors responsible for Dwarf-bunched is either identical with the blackarm-resistance gene $\mathbf{B_1}$, or else is so closely linked with it that the two may be treated as one for practical plant-breeding purposes. It is for this reason that the present paper has been included in the series on the genetics of blackarm resistance.

Previous work

The resistance to blackarm of Uganda B31 was shown by Knight & Clouston (1939) to be due to two dominant cumulative factors, $\mathbf{B_1}$ and $\mathbf{B_2}$, together with a modifier complex. By repeated backcrossing to Sakel these factors were separated from their modifier complex, and it was demonstrated that $\mathbf{B_1}$ confers weak resistance (grade '10·1'), whereas $\mathbf{B_2}$ is a strong factor giving grade '7' resistance ('0' represents immunity and '12' full susceptibility).

Knight & Clouston (1941) described the nature of blackarm-resistance inheritance in the cross Uganda B31 × 514, and they inferred that 514 contains no modifiers for $\mathbf{B}_1$ though it possesses $\mathbf{B}_2$ modifiers. 514 contains no major blackarm-resistance factors and shows the grade '10' symptoms typical of 'fully susceptible' American types.

In the same paper it was stated that 'crosses between Sakel heterozygous for factor $\mathbf{B}_1$ and 514 yield, in F_1, a 1 : 1 ratio of grades "10·1" to "12..."'. Subsequent results, obtained during the present investigation into the genetics of Dwarf-bunched, have shown that the partially resistant and susceptible classes usually overlap, so that $\mathbf{B}_1\mathbf{b}_1$ and $\mathbf{b}_1\mathbf{b}_1$ are not distinguishable with certainty on the genotypic background of the F_1.

Description of strains

514 is a vigorous American Upland (*G. hirsutum* L.) type which grows to an average height of over 4 ft. at Shambat.* It was derived, by selection, from the Sudan 'Pump Scheme Strain' which itself originated as an importation of Nyasaland Upland.†

XA 129, like 514, was obtained by selection from Pump Scheme Strain.

Uganda B31 is an American Upland type which was imported from Uganda. B31, at Shambat, shows somewhat stunted growth and does not usually average more than 2–2½ ft. in height.

Uganda SP84 is a bulk introduction from Uganda.

511D was bred in the Sudan by selection from Uganda SG85.

Deltapine is a bulk introduction from the Delta and Pineland Co., U.S.A.

513 was bred from an introduction of Punjab Early Strain, an American Upland type from India.

Sakel (*G. barbadense* L.) is a commercial type introduced from Egypt. Three types were used, viz. NT2, a tall 'leggy' variety, X1730, a shorter more 'bushy' type, and Domains Sakel proper. All three types though very distinct commercially are of Sakel origin, and for the purposes of this paper have been indiscriminately called Sakel.

Description of Dwarf-bunched

Pl. 5, fig. 1*a*, shows typical Dwarf-bunched plants and Pl. 5, fig. 1*b*, illustrates the appearance of such plants after removing all leaves. The leaves are only slightly reduced in size, whilst flowers and bolls are more or less normal. The stems and branches show excessively short inter-nodes so that the plants have a rounded, bunched-up appearance. Dwarfs grow to a height of about 8 or 10 in., whereas the normal height for 514 at Shambat is over 4 ft.

The genetics of Dwarf-bunched

Crosses between 514 *and Uganda B*31

F_1 of 514 × *Uganda B*31

The F_1 showed normal growth, the plants averaging about the same height as 514.

F_2 of 514 × *Uganda B*31

In the F_2 of 514 × B31 a number of dwarfed plants appeared. These were difficult to classify with certainty, as the F_2, in general, showed considerable variation in height and the dwarfs themselves also varied. The number of normal plants as compared with dwarfs

* Shambat is in the immediate vicinity of Khartoum, in the Northern Sudan.

† The origin of Nyasaland Upland and also of Uganda varieties is given in greater detail by Nye & Hosking in Tothill (1940).

approximated to a 13 : 3 ratio (Table 1), suggesting that normality is dependent on the presence of either of two duplicate factors, one giving an intermediate heterozygote and the other dominant.

If it be assumed that the Dwarf-bunched phenotype comprises the double recessive $\mathbf{d_a d_a d_b d_b}$ genotype with the addition of the heterozygous class $\mathbf{d_a d_a D_b d_b}$, then the F_2 of 514 and B31 (Table 1) would give the following ratio:

$$\left.\begin{array}{l} 1\mathbf{d_a d_a d_b d_b} \\ 2\mathbf{d_a d_a D_b d_b} \end{array}\right\} \text{3 Dwarf-bunched plants}$$

$$\left.\begin{array}{l} 1\mathbf{D_a D_a d_b d_b} \\ 2\mathbf{D_a d_a d_b d_b} \\ 4\mathbf{D_a d_a D_b d_b} \\ 2\mathbf{D_a D_a D_b d_b} \\ 1\mathbf{d_a d_a D_b D_b} \\ 2\mathbf{D_a d_a D_b D_b} \\ 1\mathbf{D_a D_a D_b D_b} \end{array}\right\} \text{13 Normal plants}$$

The rather poor agreement between the ratio obtained and the 13 : 3 ratio expected on this basis may be ascribed to the variability of the $\mathbf{d_a d_a D_b d_b}$ plants of the F_2.

Table 1. F_2 *of* 514 × *Uganda B*31

Family no.	Actual		Expected (13 : 3)		
	Normal	Dwarf	Normal	Dwarf	χ^2
XHA 1434	626	151	631·3	145·7	0·24
XHA 1634	401	96	403·8	93·2	0·10
XHA 1734	73	15	71·5	16·5	0·17
XHA 1834	306	69	304·7	70·3	0·03
XHA 1934	181	57	193·4	44·6	4·22
XHA 2034	72	25	78·8	18·2	3·14
XHA 2134	142	34	143·0	33·0	0·04
XHA 2234	166	58	182·0	42·0	7·50
Totals	1967	505	2008·5	463·5	15·44

$P = 0{\cdot}05$ (approx.).

In three subsequent sowings of this F_2 it was found to be impossible to grade the plants into Normals and Dwarfs with any accuracy owing to the very wide variation of the intermediate group. The most one could say was that in each of these F_2's about a quarter of the population was of intermediate or dwarf type and the remainder normal.

Dwarf-bunched × 514

A pure-breeding Dwarf-bunched family originally obtained from the F_2 of the backcross 514 × (B31 × 514) was crossed with 514. The F_1 of this cross showed normal growth and the F_2 could be classified, with no possibility of confusion, into two clear-cut groups, viz. Normal growth and Dwarf-bunched (Table 2).

Table 2. *Classification of* F_2 *of Dwarf-bunched* × 514

Family no.	Actual		Expected (3 : 1)		
	Normal	Dwarf	Normal	Dwarf	χ^2
BA 431/40	353	100	339¾	113¼	2·07
BA 432/40	324	105	321¾	107¼	0·06
BA 433/40	189	59	186	62	0·19
Totals	866	264	847½	282½	2·32

P lies between 0·5 and 0·6.

Self-bred seed of these Dwarf-bunched plants yielded dwarfs only, showing that these plants were all homozygous for the character, i.e. $\mathbf{d_a d_a d_b d_b}$.

The 3 : 1 ratio of Normals to Dwarfs obtained in the F_2 of Dwarf-bunched × 514 shows that Dwarf-bunched differs from 514 in a single recessive factor, $\mathbf{d_a}$. Thus B31 would be of $\mathbf{d_a d_a D_b D_b}$ genotype and 514 would be $\mathbf{D_a D_a d_b d_b}$. A Dwarf-bunched plant ($\mathbf{d_a d_a d_b d_b}$) crossed with 514 would give $\mathbf{D_a d_a d_b d_b}$ which would show normal growth. In F_2 the ratio 1 $\mathbf{d_a d_a d_b d_b}$: 2 $\mathbf{D_a d_a d_b d_b}$ + 1 $\mathbf{D_a D_a d_b d_b}$ would be obtained, i.e. one Dwarf-bunched to three Normals.

Dwarf-bunched × Uganda B31

The F_1 of Dwarf-bunched × B31 was mainly of intermediate habit but ranged from plants almost indistinguishable from the Dwarf-bunched parent to plants approaching the height of Uganda B31. It is evident, therefore, that this heterozygote, which, on a two-factor hypothesis, must be $\mathbf{d_a d_a D_b d_b}$, lacks dominance.

In F_2, although the presence of dwarfs was obvious, it was impossible to count them and to classify the families into Dwarf-bunched, Intermediate and Normal. This difficulty was not due solely to the variability of the Intermediate group, since, apart from variation, which was considerable, the families were grown late on very poor soil with the result that B31, never a robust type at Shambat, was itself very stunted.

The cross Uganda B31 × 513

Uganda B31 crossed with 513 gave a normal F_1 and no dwarf types in F_2.

The cross Uganda B31 × Gambia Native (G. hirsutum *var.* punctatum)

Uganda B31 crossed with Gambia Native gave a normal F_1, but Dwarf-bunched plants appeared in F_2. Again no clear-cut ratio was observed owing to the variability of the intermediate group, and the most one could say was that in a total F_2 progeny of 406 plants, not less than 19 true dwarfs were present. A repeat of this, next season, gave an F_2 progeny consisting of 13 true dwarfs to 103 plants ranging from normal to nearly dwarf. Adding these two seasons' results together gives 490 normal to 32 true dwarfs, in excellent agreement with the ratio of 489·4 : 32·6 expected on a 15 : 1 basis.

Crosses between $\mathbf{B_1 B_1}$ *Sakel and* 514

Crosses were made between Sakel containing $\mathbf{B_1}$ and 514. This blackarm-resistant Sakel was obtained from the fifth Sakel backcross of Uganda B31 × Sakel.

The F_1 of $\mathbf{B_1 B_1}$ Sakel × 514 showed normal growth, but in F_2 a number of typical Dwarf-bunched plants appeared. The F_2 could not be classified into definite groups because of the great variation in height commonly found in such *barbadense* × *hirsutum* F_2's, and, although typical Dwarf-bunched plants were present, there was a more or less complete gradation from dwarfs to the shorter 'normals'. As a check, however, crosses had been made between $\mathbf{b_1 b_1}$ Sakel (also of fifth backcross origin) and 514 and, though these also showed great variation in height in F_2, no Dwarf-bunched plants were present.

In the following seasons this experiment was repeated, using first $\mathbf{B_1 B_1}$ Sakel derived from the seventh Sakel backcross of Uganda B31 × Sakel and, later, ninth, eleventh and twelfth backcross material. In the F_2's of all these $\mathbf{B_1 B_1}$ Sakel × 514 hybrids, a number of Dwarf-bunched plants again appeared.

Since, even after thirteen crosses of $\mathbf{d_a B_1}$ with Sakel $\mathbf{D_a b_1}$, the two genes $\mathbf{d_a}$ and $\mathbf{B_1}$ had not been separated, it is evident that they must be closely linked. Indeed, it might well

be argued that they are identical, and that the action of $\mathbf{d_a}$ in producing Dwarf-bunched in conjunction with $\mathbf{d_b}$ is merely a pleiotropic effect of $\mathbf{B_1}$.

Crosses between $\mathbf{B_1B_1}$ *Sakel and various cotton varieties*

In the following crosses the male parent was, in each case, BAR 2/8. This variety is a Sakel type homozygous for $\mathbf{B_1}$. It was obtained by selfing out $\mathbf{B_1B_1}$ plants from seventh Sakel backcross material of Uganda B 31 × Sakel origin.

BAR 2/8 was crossed with a number of American Upland strains with the following results:

G. hirsutum parent	F_1	F_2
Uganda SP 84	Normal	One progeny contained Dwarf-bunched plants, a second contained none
511 D	,,	No Dwarf-bunched
XA 129	,,	Contained Dwarf-bunched
514	,,	Contained Dwarf-bunched
Deltapine	,,	One progeny contained Dwarf-bunched, a second contained none

From these crosses, and those reported earlier, it is clear that the gene $\mathbf{d_b}$ is present in Gambia Native (*G. hirsutum* var. *punctatum*), 514, Uganda SP 84, XA 129 and Deltapine, but that it is not present in all *hirsutum* cottons. It is absent, also, from the three Sakel (*barbadense*) varieties: Domains Sakel, NT 2 and X 1730, since transferences of $\mathbf{B_1}$ to these varieties have been made with complete success and have produced no sign of dwarfing.

Transference of Dwarf-bunched from G. hirsutum *to* G. barbadense

When the close connexion between the character Dwarf-bunched and the blackarm-resistance gene $\mathbf{B_1}$ was realized it seemed probable that the knowledge could be utilized in checking the genotype of Sakel strains to which $\mathbf{B_1}$ had been transferred. Clearly this check would be superfluous in a Sakel containing $\mathbf{B_1}$ alone, since spraying with blackarm disease would at once disclose the presence of this factor. Where, however, $\mathbf{B_1}$ was combined with $\mathbf{B_2}$ in a single strain, the grade of resistance, alone, proved to be no certain test of the presence of $\mathbf{B_1}$ since this gene does not greatly add to the resistance of $\mathbf{B_2}$ and there is a considerable overlap between the $\mathbf{B_1B_2}$ and $\mathbf{b_1B_2}$ phenotypes in a Sakel background.

As a test for the presence of $\mathbf{B_1}$ in a variety, crosses could be made with Dwarf-bunched. Plants homozygous for $\mathbf{B_1}$ would then yield F_1 seed from which intermediate Dwarf-bunched plants would grow.

It was felt that, for such check-crossing, the clearest results would be obtained if the character Dwarf-bunched were first transferred to a Sakel background.

Accordingly, a start was made using homozygous dwarfs selfed out from an F_2 progeny of 514 × $\mathbf{B_1B_1}$ Sakel. One of these dwarfs was backcrossed to $\mathbf{B_1B_1}$ Sakel yielding a progeny of semi-dwarfs which showed considerable variation.

In such a transference, clear segregation would not be expected, since the $\mathbf{D_b}$ heterozygote has been shown to be intermediate and to overlap the normal and the homozygous dwarf classes. In fact, although in each backcross generation it was possible to choose, with confidence, semi-dwarfs as parents for the next generation, it was never possible to be certain of the upper limit of the semi-dwarf class. Approximate classifications were made and gave the distributions shown in Table 3. There was a similar uncertainty in the classification of progenies obtained on selfing intermediate plants in the second, third and fourth backcrosses. Samples of the data obtained are given in Tables 4 and 5.

One noticeable thing in these various backcrosses to $\mathbf{B_1B_1}$ Sakel strains was that the intermediate Dwarf-bunched ($\mathbf{d_a d_a D_b d_b}$) lost much of its similarity to Dwarf-bunched and became more 'normal' in appearance, though still ranging in odd plants almost down to

Table 3. *Summary of results obtained by backcrossing* 514 *to* $\mathbf{B_1B_1}$ *Sakel*

	Normal	Intermediate
2nd backcross	47	48
3rd backcross	20	18
4th backcross	10	8
Totals	77	74

the full Dwarf-bunched. The main difference in the later 'Sakel' backcrosses between intermediates and normals was that intermediate plants were much more monopodial and 'bushy' than the normals.

Eight intermediate plants selfed in the second 'Sakel' backcross gave the distributions shown in Table 4.

Table 4. F_2 *of* 514 *backcrossed twice to* $\mathbf{B_1B_1}$ *Sakel*

Family no.	Dwarf + Intermediate	Normal	Expected 3 : 1	Totals	χ^2
BA 271/43	207	60	$200\frac{1}{4}$: $66\frac{3}{4}$	267	0·91
BA 272/43	93	26	$89\frac{1}{4}$: $29\frac{3}{4}$	119	0·63
BA 274/43	33	18	$38\frac{1}{4}$: $12\frac{3}{4}$	51	2·88
BA 275/43	62	37	$74\frac{1}{4}$: $24\frac{3}{4}$	99	8·08
BA 276/43	196	97	$219\frac{3}{4}$: $73\frac{1}{4}$	293	10·27
BA 278/43	44	27	$53\frac{1}{4}$: $17\frac{3}{4}$	71	6·43
BA 280/43	157	70	$170\frac{1}{4}$: $56\frac{3}{4}$	227	4·13
Totals	792	335	$845\frac{1}{4}$: $281\frac{3}{4}$	1127	33·33

P is less than 0·001.

In the F_2 of the fourth backcross to $\mathbf{B_1B_1}$ Sakel the progenies were classified into three groups 'Normal', 'Doubtful' and 'Dwarf plus Intermediate' (Table 5).

Table 5. F_2 *of* 514 *backcrossed four times to* $\mathbf{B_1B_1}$ *Sakel*

Family no.	Dwarf + Intermediate	Doubtful	Normal
BA 641/45	225	40	44
BA 642/45	86	8	52
BA 643/45	91	13	80
BA 644/45	106	6	38
BA 645/45	159	8	119
BA 646/45	166	12	62
Totals	833	87	395
Expected (3 : 1)	986·25	—	328·75

That the uncertainty of the classification is due to the variability of a single heterozygous class ($\mathbf{d_a d_a D_b d_b}$) is shown by the results of the detailed examination of family BA 646/45. The data in Table 5 were taken early in the season: this family was later re-examined by two observers, and the distinction between the classes was found to have improved with age. It was found that the family could be grouped with some certainty into 60 Dwarfs, 106 Intermediates, 12 Doubtfuls and 62 Normals. Adding the Doubtfuls to the Intermediates gives 60 : 118 : 62—a close approximation to expectation on a 1 : 2 : 1 basis.

The relationship between $\mathbf{d_a}$ *and the blackarm-resistance gene* $\mathbf{B_1}$

A Dwarf-bunched plant in the F_2 of a second Sakel backcross was crossed with $\mathbf{b_1b_1}$ Sakel. All the F_1 plants showed normal growth; progenies of nine of these plants gave the distributions shown in Table 6.

Table 6. F_2 *of third Sakel backcross*

Family no.	Normal		Doubtful		Dwarf + Intermediate	
	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible
BA 647/45	86	28	13	2	27	—
BA 648/45	115	59	20	1	22	—
BA 649/45	90	41	12	2	26	—
BA 650/45	131	46	9	—	30	—
BA 651/45	89	56	10	—	24	—
BA 653/45	119	45	7	2	36	—
BA 654/45	130	56	18	1	38	—
BA 655/45	156	91	14	1	61	—
BA 656/45	85	62	13	1	28	—
Totals	1001	484	116	10	292	—

The close linkage of $\mathbf{B_1}$ and $\mathbf{d_a}$ is amply demonstrated in this material. Among 292 plants classified as Dwarf or Intermediate there was not a single susceptible plant.

DISCUSSION

From the standpoint of the applied geneticist, the importance of the character Dwarf-bunched lies in the close linkage which obtains between the blackarm-resistance gene $\mathbf{B_1}$ and the factor $\mathbf{d_a}$. In the first place it would obviously be of value to free $\mathbf{B_1}$ from its deleterious component $\mathbf{d_a}$ in order to render this resistance gene 'safe' for use in all crosses. There appears to be little hope of doing this, since linkage of $\mathbf{d_a}$ and $\mathbf{B_1}$ has been shown to be close. Indeed, during the course of a large programme of crossing Dwarf-bunched with blackarm-susceptible types no susceptible Dwarf-bunched or susceptible Intermediate plant ever appeared.

To the pure geneticist the main point of interest about Dwarf-bunched is that the duplicate genes $\mathbf{D_a}$ and $\mathbf{D_b}$ have different dominance reactions. On Fisher's (1930) theory, dominance is dependent on the genotypic background rather than on the gene itself. These duplicate factors do not conform to this theory in that, on the one genetic background, $\mathbf{D_a}$ is fully dominant whilst $\mathbf{D_b}$ lacks dominance. In the case of $\mathbf{D_a}$, therefore, dominance is a function of the gene itself.

SUMMARY

In the F_2 of a cross between two American Upland (*G. hirsutum*) types, Uganda B31 and the Sudan variety 514, a number of markedly dwarfed, 'bunched-up' plants appeared. Investigation showed that normality as opposed to 'Dwarf-bunched' depends on the presence of either of two duplicate genes, one dominant and the other giving an intermediate heterozygote. These genes have been called $\mathbf{D_a}$ and $\mathbf{D_b}$, the former deriving from 514 and the latter from Uganda B31. 514 is of $\mathbf{D_aD_ad_bd_b}$ genotype, Uganda B31 is $\mathbf{d_ad_aD_bD_b}$, Dwarf-bunched is $\mathbf{d_ad_ad_bd_b}$, and the heterozygote $\mathbf{d_ad_aD_bd_b}$ shows considerable variability with a range from dwarf to normal.

The gene $\mathbf{d_b}$ occurs in Gambia Native (*G. hirsutum* var. *punctatum*) and in the American Upland varieties Uganda SP84, XA129 and Deltapine, but it was not present in all Upland varieties examined.

The gene $\mathbf{d_a}$ is closely linked with (or possibly identical with) the blackarm-resistance gene $\mathbf{B_1}$. Since *G. barbadense* types are of $\mathbf{D_aD_aD_bD_b}$ composition, $\mathbf{B_1}$ can be utilized in conferring blackarm resistance on this group. Its use within the *G. hirsutum* group is fraught with the danger of producing Dwarf-bunched types as the end-product.

I am greatly indebted to Mr J. B. Hutchinson for useful suggestions and discussions made during the writing of this paper.

REFERENCES

Fisher, R. A. (1930). *The Genetical Theory of Natural Selection.* Oxford University Press.

Knight, R. L. & Clouston, T. W. (1939). The genetics of blackarm resistance. I. *J. Genet.* **38**, 133–59.

Knight, R. L. & Clouston, T. W. (1941). The genetics of blackarm resistance. II and III. *J. Genet.* **41**, 391–409.

Tothill, J. D. (Editor) (1940). *Agriculture in Uganda.* Oxford University Press.

EXPLANATION OF PLATE 5

Fig. 1*a*

Two fully developed Dwarf-bunched plants.

Fig. 1*b*

Two fully developed Dwarf-bunched plants from which all leaves have been removed.

Fig 1*a*

GENETICS OF RED CLOVER (*TRIFOLIUM PRATENSE* L.) COMPATIBILITY

II. (*a*) HOMOZYGOUS SELF-STERILE S_xS_x GENOTYPES OBTAINED AS A RESULT OF PSEUDO-FERTILITY; (*b*) SELF-FERTILITY

DATA COLLECTED BY THE LATE R. D. WILLIAMS, M.Sc.
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I. INTRODUCTION

Previous investigations have demonstrated that the species *Trifolium pratense* is almost completely self-sterile and very highly cross-fertile, and that sterility is conditioned by an extensive allelomorphic series of oppositional S factors. No evidence has as yet been obtained to indicate the presence of factors capable of modifying the effect of the sterility factors, but instances of pseudo-fertility are met with where plants upon being selfed are capable of effecting a very low percentage of fertilization and seed-setting. Pseudo-fertility in red clover has been classified as true sterility (Williams & Silow, 1933) where an occasional pollen tube, even though it carries an S factor similar to that borne by the maternal stylar tissue, is capable of traversing the whole length of the style and effecting fertilization. On the hypothesis that sterility in red clover is of the simple oppositional factor type it would be expected that three genotypes would be obtained as a result of pseudo-fertility—two of these genotypes would be homozygous for the sterility factors. It has already been shown (Williams & Silow, 1933) that homozygous genotypes are obtained in this way, and further data are presented in this paper on the identification and crossing of four homozygous sterility genotypes—S_1S_1, S_2S_2, S_3S_3 and S_4S_4—obtained as a result of pseudo-fertility from incompatible matings.

In addition to the sterility allelomorphs $S_1, S_2, \ldots, S_n$, a self-fertility allelomorph (S_f) has been isolated in one individual red clover plant 344(1) 263. This allele, which is believed to have arisen as a mutation in the S locus, effects full fertility when present in either the

homozygous or heterozygous condition. Indirect evidence shows that the self-fertility factor originated in a plant which was heterozygous for the S factors and of the constitution S_fS_a. On the basis of the assumption that only S_f pollen functions on selfing a plant of the constitution S_fS_a, all the progeny of selfing would be self-fertile—being S_fS_f or S_fS_a in constitution.

A number of investigators who have studied self-fertility in species which also exhibit self-sterility report that the behaviour of the sterility and fertility factors is frequently modified by the action of one or more genes which may be independent of, or linked with, the S locus. Brieger (1927), from a study of a self-fertile plant in *Nicotiana Sanderae*, concluded that self-fertility was conditioned by a factor P which was linked with the S factor and modified its action. East (1932) concluded from crosses between *N. Langsdorffii* and *N. Sanderae* that the self-fertility factor S_f behaved as an allele to the self-sterility factors, S_1, S_2, etc., but that besides there were three additional alleles, A_1, A_2 and A_3, which were independent of the S locus and capable of reducing to a variable degree the inhibitory actions of certain of the S alleles. Anderson & Winton (1931) assumed the presence of two independent modifying factors R and R_1 to explain the behaviour of a self-sterile plant of *N. alata* when crossed as male with a self-fertile plant of *N. Langsdorffii*. Gruber (1932), working with *Antirrhinum glutinosum*, postulated the presence of a fertility factor P which was linked with the S factors; S_aS_bpp was assumed to be self-sterile, and S_aS_bPp self-fertile. Kakizaki (1930) explained his work on *Brassica oleracea* as being due to the action of two separate allelomorphic series of factors S and T. According to Kakizaki, similar S factors in the pistil and in the pollen grains effected the inhibition of the pollen tubes, but the S factors were epistatic to the second allelomorphic series of factors T_1 and T_2, which favoured fertilization. Beatus (1934), adopting Lawrence's view that *Cardamine pratensis* is an autotetraploid, explained compatibility in the species on somewhat similar lines to those formulated by Kakizaki. In *C. pratensis* two allelomorphic series of sterility factors S and Z, and two allelomorphic series of fertility factors T and F, were assumed to be involved. Wergin (1936), studying a diploid cultivated *Petunia*, assumed the presence of H and h modifying fertility factors in addition to an (S_f) factor of the series S_1, S_2, S_3, ..., S_n. Self-fertility was effected by the S_f factor and by S_1S_2 genotypes when present in association with hh in the homozygous condition. Half-fertility was dependent on the heterozygote Hh, while full sterility was conditioned only by the oppositional action of the S factors in conjunction with HH. Stout (1938) also presents results with the *Petunia* 'Rosy Morn' as evidence of two allelomorphic series of factors—an S series effecting sterility and an F or T series effecting fertility.

II. Self-sterility in red clover

A considerable amount of evidence (Williams, 1925, 1931; Williams & Silow, 1933) has been brought forward indicating that self- and cross-sterility in red clover are determined by a single allelomorphic series of factors acting oppositionally, and agreeing with the oppositional factor hypothesis propounded by Prell (1921) and East & Mangelsdorf (1925). Ratios expected on the oppositional factor hypothesis have been repeatedly obtained, and the apparent regularity of the behaviour of red clover in this respect is to some extent explicable by the fact that it is a diploid species with 14 chromosomes. Progenies resulting from compatible out-crosses, where a sufficiently large number of

plants have been tested, consist of either two or four intra-sterile, inter-fertile groups according to the genetic constitution of the parents.

The results of tests of compatibility in family 5843(1) are discussed below. This F_1 family was derived from a cross between two ordinary commercial plants of red clover, and twenty sibs taken at random were each pollinated by a sib-tester representing each genotypic group present in the family. The percentage seed set per 100 florets pollinated in the sib crosses is set out in Table 1.

Table 1. *Analysis into four genotypic classes of twenty F_1 progeny of a cross $S_aS_b \times S_xS_y$, according to their percentage fertility relationships*

	♀																			
	S_aS_x										S_aS_y			S_bS_x			S_bS_y			
♂ Sibs ...	1	2	3	4	6	9	10	16	18	19	8	12	13	5	14	17	7	11	15	20
S_aS_x no. 18	0	0	0	0	0	0	0	0	—	2	56	63	72	77	70	74	75	67	76	68
S_aS_y no. 13	86	106	76	72	68	76	38	82	60	86	0	0	—	55	107	86	80	72	100	77
S_bS_x no. 14	97	85	64	100	70	36	63	63	78	66	47	53	83	0	—	0	—	53	50	65
S_bS_y no. 11	81	70	76	47	61	54	53	81	72	52	58	83	56	44	80	91	0	—	0	0

— = Not crossed.

	S_aS_x		S_aS_y		S_bS_x		S_bS_y
Expected ratio	5	:	5	:	5	:	5
Observed ratio	10	:	3	:	3	:	4

$\chi^2 = 6{\cdot}8$; $P = 0{\cdot}10$–$0{\cdot}05$.

Since the two parent plants used in this cross were entirely unrelated, they were expected to have different S alleles—S_aS_b and S_xS_y. On the basis of this assumption the F_1 progeny would be expected to belong to one of four different genotypic classes—S_aS_x, S_aS_y, S_bS_x and S_bS_y. As shown in Table 1, these four classes were actually obtained, and although the observed ratio 10 : 3 : 3 : 4 deviates rather widely from the expected equality, the values of P for these distributions indicates that probably the deviations are not significant.

III. PSEUDO-FERTILITY

It has already been reported (Williams & Silow, 1933) that in a very low percentage (0·10) of cases it is found that certain pollen tubes carrying the same S factor as may be carried by the female parent are able to penetrate the full length of the stylar tissue and effect fertilization. The effect of this phenomenon of pseudo-fertility is occasionally seen in otherwise self- and cross-sterile pollinations where a few viable seeds are sometimes produced. The percentage of viable seeds in such pseudo-fertile crosses seldom exceeds 2–3, although in some instances it has amounted to 5 or 6. The progeny produced as a result of pseudo-fertilization from an individual bearing the factors S_1 and S_2 would be expected to consist of the genotypic classes S_1S_1, S_1S_2 and S_2S_2 in the ratio of 1 : 2 : 1. With a view to demonstrating that certain S factors are occasionally capable of traversing stylar tissue in spite of the inhibitory action of the same gene, and in order to isolate certain genotypes homozygous for the S factor, tests were conducted with several groups containing small numbers of plants obtained as a result of pseudo-fertility, and the results are discussed below.

IV. Isolation of homozygous S_xS_x genotypes obtained through pseudo-fertility

(1) S_1S_1 *genotype*

(*a*) *Origin.* This genotype originated as a result of crossing two sister plants in family 628(1). Two sister plants 628(1) 1 and 628(1) 2 were reciprocally crossed, and from 256 florets pollinated on 628(1) 1 no seed was obtained, while plant 628(1) 2 when used as female produced only three seeds. Both these plants, in view of their percentage cross-fertility, can be regarded as possessing like pairs of sterility factors, and the very low seed set (1·50 %) obtained when plant 628(1) 2 was used as female is attributed to pseudo-fertility.

From the three seeds obtained, only two plants originated, and these were tested for their self- and cross-fertility relationships. The results of these tests are presented in Table 2.

Table 2. *The self- and cross-fertility relationships of two sister plants* (628(2) 2*Ma* (1*P*) *and* (2*P*)) *obtained from the cross* 628(1) 1 ♂ × 2 ♀ *as a result of pseudo-fertility*

		Percentage fertility. Male			
		Parents S_1S_x		F_2 sister plants	
				S_1S_x	S_1S_1
		628(1) 1	628(1) 2	628(2) 2Ma (1P)	628(2) 2Ma (2P)
Female	Parents:				
	S_1S_x: 628(1) 1	0	0	0	0
	628(1) 2	1·5	0	0	0
	F_2 sister plants:				
	S_1S_x, 628(2) 2Ma (1P)	0	0	0	0
	S_1S_1, 628(2) 2Ma (2P)	35	63	74·6	0

Table 2 shows that the sister plant 628(2) 2Ma (1P) was reciprocally cross-sterile with both its parents, but that 628(2) 2Ma (2P) was cross-sterile when used as male and cross-fertile when used as female with both its parents as well as with its sister plant 628(2) 2Ma (1P). These results indicate that both parents and progeny (1P) carried like sterility factors, and they have accordingly been assigned the genetic constitution S_1S_x. The fertility relationship of (2P) can only be explained by assuming that it was homozygous for the S_1 factors and the genetic designation S_1S_1 has been adopted.

(*b*) *Compatibility of progeny resulting from the cross* 628(2) 2*Ma* (1*P*) ♂ × (2*P*) ♀— ($S_1S_x \times S_1S_1$). It will be seen from Table 2 that the cross 628(2) 2Ma (1P) ♂ × (2P) ♀ gave a seed set of 74·6 %, and on the assumption that (1P) was of the constitution S_1S_x, and (2P) S_1S_1, all the progeny resulting from such a cross should be S_1S_x and therefore all inter-sterile.

In order to test the correctness of this assumption, thirty-five sibs were raised from the above cross and their fertility relationships with four tester sibs were determined. The results of the tests are set out in Table 3.

In these crosses sibs 4, 23, 2 and 3 were used as testers and the direction of the crosses was as indicated in Table 3. All the sibs, with the exception of no. 36, gave no seed when pollinated by the testers. Sib 36 gave a seed set of only 2·7 % when pollinated by sib 3, and this is within the limits of seed setting obtained through pseudo-fertility in certain incompatible crosses. Tester sibs 4 and 23 were out-crossed in order to ascertain that they were effectively male-fertile in compatible crosses. In these tests the tester plants were

crossed reciprocally with an unrelated plant, and a seed set of from 67 to 77% was obtained from all the crosses, thus proving that the testers were functionally fully fertile.

These results show that all the progeny obtained from this cross bore like sterility factors as would be expected if 628(2) 2Ma (2P) were homozygous for the S factors.

(c) *Back-crossing of progeny obtained from crossing* 628(2) 2*Ma* (2*P*) S_1S_1 *with unrelated plants*—S_xS_y. The series of alleles concerned with sterility in red clover is a very extensive one, and it but rarely happens therefore that two unrelated plants are cross-sterile. In a further attempt to determine the range of allelomorphic factors governing sterility in red

Table 3. *The fertility relationships of thirty-five sibs* (S_1S_x) *obtained from the cross* 628(2) 2*Ma* 1*P* ♂ × 2*P* ♀ (S_1S_x ♂ × S_1S_1 ♀)

		S_1S_x sibs, ♀																
	No.	1	2	3	6	7	8	9	11	12	15	23	13	14	16	17	18	20
S_1S_x tester sibs, ♂	4	0	0	0	0	0	0	0	0	0	0	—	—	—	—	—	—	—
	23	—	—	—	—	—	—	—	—	—	—	0	0	0	0	0	0	0
	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

		S_1S_x sibs, ♀																
	No.	21	24	25	26	27	28	29	30	31	33	34	35	36	37	38	39	40
S_1S_x tester sibs, ♂	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	23	0	0	0	0	0	—	—	—	—	—	—	—	—	—	—	—	—
	2	—	—	—	—	—	0	0	0	0	0	—	—	—	—	—	—	—
	3	—	—	—	—	—	—	—	—	—	—	0	0	⊕	0	0	0	0

0 = full sterility. ⊕ = very slight pseudo-fertility. — = not crossed.

clover, a number of unrelated plants were crossed with the homozygous S_1S_1 plant 628(2) 2Ma (2P), and on the assumption that the unrelated plants each possessed two different sterility factors S_x and S_y, then each F_1 family should be of the constitution S_1S_x and S_1S_y (see this *Journal*, p. 72).

In order to ascertain that the S factor introduced from the common parent 628(2) 2Ma (2P) was in each case an S_1, one F_1 progeny of these crosses was back-crossed as female and occasionally as male to the S_1S_1 parent. The results of these back-crosses are shown in Table 4.

Table 4. *The percentage fertility results obtained by back-crossing* S_1S_x *or* S_1S_y F_1 *progenies with the homozygous* S_1S_1 *parent*

		Female: S_1S_x or S_1S_y										S_1S_1
Male		1608(1) 1	1614(1) 2	1615(1) 3	1616(1) 2	1618(1) 6	1619(1) 5	1621(1) 3	1622(1) 6	1623(1) 5	1625(1) 5	628(2) 2Ma (2P)
S_1S_1	628(2) 2Ma (2P)	0	0	0	0	0	0	3·3	0	0	0	—
S_1S_x or S_1S_y	1613(1) 5	—	—	—	—	—	—	—	—	—	—	56·4
	1627(1) 4	—	—	—	—	—	—	—	—	—	—	59·5

From Table 4 it will be seen that 628(2) 2Ma (2P)—S_1S_1 when used as male on a plant selected from each of ten F_1 families of the constitution S_1S_x or S_1S_y yielded no seed except in one cross, where owing to pseudo-fertility a seed set of 3·3% was obtained. When the S_1S_1 parent was used as female with two of the F_1 plants, S_1S_x or S_1S_y, a seed

set of 56·4 and 59·5 % was obtained. This latter result was in accordance with expectation, since each F_1 progeny possessed an S_x or S_y factor which was derived from the unrelated parent. Pollen grains carrying either S_x or S_y factors functioned normally on the style of the S_1S_1 parent.

The results of back-crossing these F_1 plants show that each one of the progeny contained an S_1 factor, thus furnishing additional proof that the parent 628(2) 2Ma (2P) was homozygous for the sterility factors.

(2) S_2S_2 *genotype*

(*a*) *Origin.* This genotype arose from a reciprocal cross between two sibs (nos. 4 and 20) in family 393(1). From 191 florets pollinated, on 393(1) 20, no seed was obtained, while a seed set of 2·3 % was obtained as a result of pseudo-fertility from 222 florets pollinated on sib 4. From the five seeds obtained in this way two plants were raised, and on the assumption that the two parents possessed like sterility factors and that the seeds arose as a result of pseudo-fertility, the progeny could belong to any one of three sterility groups. Assuming both the parents to be of the constitution S_2S_x, the progeny would be S_2S_2, S_2S_x or S_xS_x. Since one of the progeny—393(2) 4Ma (2P)—raised from the cross is proved hereunder to be homozygous for the S factor, it is proposed to designate it by the genetic formula S_2S_2.

As a first step in determining the genetic constitution of 393(2) 4Ma (2P), it was reciprocally back-crossed to one of its parents—393(1) 4. From 202 florets pollinated on 393(1) 4 no seed was obtained, while when 393(2) 4Ma (2P) was used as female forty-eight seeds were obtained from the ninety-eight florets pollinated—a seed set of 49 %. Both 393(1) 4 and 393(2) 4Ma (2P) when self-pollinated were completely sterile. On the oppositional factor hypothesis this result indicates that 393(2) 4Ma (2P) was homozygous for the S factor.

(*b*) *The genetic constitution of sibs obtained from the cross* 393(2) 4*Ma* (2*P*) ♀ (S_2S_2) × 393(1) 4 ♂ (S_2S_x). From the compatible back-cross 393(2) 4Ma (2P) ♀ × 393(1) 4 ♂ seven sibs were raised and their inter-fertility reactions tested by crossing the other six sibs reciprocally by sib 7. All the progenies from the above cross (S_2S_2 ♀ × S_2S_x ♂) should be of the genetic constitution S_2S_x, with the exception of an occasional S_2S_2 plant which might infrequently arise as a result of pseudo-fertility.

Table 5. *The percentage fertility relationship of tester sib* 7 *obtained from the cross* 393(2) 4*Ma* (2*P*) ♀ × 393(1) 4 ♂ *when reciprocally crossed with:* (*a*) *six other sister plants;* (*b*) 393(1) 4 *parent; and* (*c*) *an unrelated plant Aa* 1904(14)

		Sister plants S_2S_x						393(1) 4	Aa 1904(14)
		1	2	3	4	5	6	S_2S_x	S_xS_y
Tester sib S_2S_x	7 ♂	0	0	0	0	0	0	0	50
	7 ♀	0	—	0	0	0	0	0	53

— = not crossed.

The results set out in Table 5 demonstrate that the tester sib 7 was reciprocally incompatible both as male and female with its six sister plants as well as with its heterozygous parent 393(1) 4. When reciprocally out-crossed to an unrelated plant (Aa 1904(14)), the tester sib gave a normal result and set seed to the extent of 50 % of the florets pollinated. Sib 7 was therefore fully functional both as male and female in compatible crosses.

These results indicate that all the seven sibs as well as their male heterozygous parent 393(1) 4 were of the same genetic constitution; as would be expected if the female parent 393(2) 4Ma (2P) was homozygous for the sterility factors.

(3) S_3S_3 *genotype*

(*a*) *Origin.* The S_3S_3 genotype was obtained from a cross between two F_1 sibs of family 604(1). A cross between the two sibs—nos. 2 and 3—proved to be incompatible, and from 270 florets pollinated on 604(1) 2 by 604(1) 3 no seed was obtained, while 254 florets on 604(1) 3 yielded only three seeds when pollinated by 604(1) 2. Two plants—604(2) 3Ma (1P) and 604(2) 3Ma (2P)—were raised from these three seeds, and if the constitution S_3S_x is assigned to the parents, the two offspring could be either S_3S_3, S_3S_x or S_xS_x.

The two sibs 604(2) 3Ma (1P) and 604(2) 3Ma (2P) when selfed gave a seed set of 2·2 and 3·2% respectively, as a result of pseudo-fertility. On reciprocally inter-crossing 604(2) 3Ma (1P) × 604(2) 3Ma (2P), a seed set of 51·4 and 1·9% was obtained from (1P) and (2P) respectively.

From these results of selfing and cross-pollinating it is evident that both plants were slightly pseudo-fertile—the percentage pseudo-fertility ranging from 1·9 to 3·2. Since the cross was fully fertile only when 604(2) 3Ma (1P) was used as female, it indicates that this plant was homozygous and that 604(2) 3Ma (2P) was heterozygous for the sterility factors. These plants have therefore been assigned the genetic constitution S_3S_3 and S_3S_x, respectively.

In order to test the compatibility of the progeny of the cross 604(2) 3Ma (1P) ♀ × 604(2) 3Ma (2P) ♂ ($S_3S_3 \times S_3S_x$), thirty-seven sibs were inter-pollinated by means of sterilized bees* in a bee-proof greenhouse. From an average of 3½ heads (about 150–200 florets) examined for seed on each sib, only one seed was obtained. Although it must be admitted that this method of testing is not entirely satisfactory since there is no means of ensuring that the cross-pollinations have actually been effected in all possible directions, the result obtained strongly suggested that the thirty-seven sibs were of the same genotype, as was expected from the assumption that 604(2) 3Ma (1P) was homozygous for the S factors.

(*b*) *Progeny of a cross between* S_3S_3 ♂ × S_xS_y ♀ (604(2) 3*Ma* (1*P*) ♂ × *unrelated plant* ♀). As part of an investigation to determine the number of allelomorphs of the S factor present in red clover, twenty-five plants selected at random from an unbred population known as English late-flowering red clover were pollinated by 604(2) 3Ma (1P). Apart from plant 17 which yielded only ten poorly developed and non-viable seeds from ninety-nine florets pollinated, and which may therefore have been carrying an S_3 gene, all the crosses were fertile. These results indicate that with the possible exception of plant 17, all the twenty-five plants carried sterility genes which were different from the one designated as S_3.

Each of the twenty-four F_1 progeny obtained by crossing S_xS_y ♀ × S_3S_3 ♂ were found by appropriate tests to fall into two different genetic classes, that is, S_3S_x or S_3S_y. One individual from each of the forty-eight genotypic classes was reciprocally back-crossed to the homozygous S_3S_3 parent 604(2) 3Ma (1P), and as these results are reported in detail elsewhere in the present issue of this *Journal* it suffices here to state that, when

* The technique of sterilizing bees for critical genetical crosses has been described in *Welsh Plant Breeding Station Bull.* Ser. H, no. 4, 1921–4, p. 44.

604(2) 3Ma (1P) was used as female, full fertility resulted from all the back-crosses, whereas when 604(2) 3Ma (1P) was used as pollinator on any one of the forty-eight genotypes S_3S_x or S_3S_y, all the crosses were sterile or only slightly pseudo-fertile.

These results indicate clearly that the forty-eight F_1 genotypes obtained by crossing 604(2) 3Ma (1P) by twenty-four unrelated plants all contained an S_3 gene, and furnish further support for the view that 604(2) 3Ma (1P) was of the genetic constitution S_3S_3.

(4) S_4S_4 *genotype*

(*a*) *Origin.* This homozygote arose in family 675(1) from a cross between two inter-sterile sibs—675(1) 3 and 4. From 200 florets only four seeds were obtained from the above cross when sib 3 was used as female, and the reciprocal cross using sib 4 as female was fully sterile even though 276 florets were pollinated. It is indicated, therefore, that sibs 3 and 4 were of the same genetic constitution and that the 2% seed set obtained on sib 3 was the result of pseudo-fertilization.

Of the four seeds so obtained only two were viable, and the two plants raised bear the numbers 675(2) 3Ma (1P) and 675(2) 3Ma (2P). Assuming that the parents were of the genetic constitution S_4S_x, then the offspring may belong to one of three genetic classes, viz. S_4S_4, S_4S_x, or S_xS_x. Since both these progeny plants are shown below to be reciprocally inter-sterile and homozygous, they have been assigned the genetic constitution S_4S_4.

(*b*) *The genetic constitution of the two progeny plants* 675(2) 3*Ma* (1*P*) *and* (2*P*). The percentage fertility results of selfing, inter-crossing and back-crossing of two progeny plants raised as a result of pseudo-fertilization in the cross 675(1) 3 ♀ × 4 ♂ are shown in Table 6.

Table 6

		S_4S_x parent 675(1) 3	S_4S_4 progeny 675(2) 3Ma (1P)	S_4S_4 progeny 675(1) 3Ma (2P)
S_4S_x parent	675(1) 3	1·5	—	—
S_4S_4 progeny	675(2) 3Ma (1P) ♂	0·0	—	—
	675(2) 3Ma (1P) ♀	31·6	0	—
	675(2) 3Ma (2P) ♂	0·0	0	—
	675(2) 3Ma (2P) ♀	44·0	0	0

— = Not crossed.

Note. Both 675(2) 3Ma (1P) and (2P) functioned normally as seed and pollen parents with unrelated plants, and gave a seed set ranging from 58·0 to 62·9%.

It is shown in Table 6 that both the parent 675(1) 3 and the progeny were self-sterile—the 1·5% seed set that resulted from selfing 675(1) 3 being the result of pseudo-fertility. When back-crossed both the progeny plants were fertile as female but sterile as male with 675(1) 3. It will also be noted that 675(2) 3Ma (1P) and (2P) were sterile when reciprocally inter-crossed.

These results clearly indicate (1) that both (1P) and (2P) were of the same genetic constitution, and (2) that they were homozygous for the sterility factors.

Genotypes in a cross 675(2) 3*Ma* (2*P*) ♂ × *Aa* 1904(14) ♀ ($S_4S_4 \times S_xS_y$). Thirteen sibs were raised from the cross 675(2) 3Ma (2P) × Aa 1904(14), and on the assumption that the plants were respectively of the genetic constitutions S_4S_4 and S_xS_y, two distinct genotypes—S_4S_x and S_4S_y—were expected in equal numbers in the progeny. This expectation

was investigated by means of appropriate crosses, the results of which are set out in Table 7.

Table 7. *The compatibility of thirteen sibs derived from the cross $S_4S_4 \times S_xS_y$ when reciprocally crossed with two tester sibs, and when reciprocally back-crossed to both parents*

		S_4S_x sibs						S_4S_y sibs						
		1	3	6	7	10	11	2	4	5	8	9	13	14
S_4S_x tester sib	1 ♀	—	0	0	—	0	0	+	+	+	—	+	+	+
	1 ♂	—	0	0	—	0	0	+	+	+	—	+	+	+
S_4S_y tester sib	2 ♀	+	—	+	+	+	+	—	0	—	0	0	0	—
	2 ♂	+	—	+	+	+	+	—	0	—	0	0	0	—
S_4S_4 parent: 675(2) 3Ma (2P)	♀	—	+	+	—	+	+	+	+	+	+	+	+	—
675(2) 3Ma (2P)	♂	0	—	0	0	0	0	0	0	0	0	0	0	—
S_xS_y parent: Aa 1904(14)	♀	+	+	+	+	+	+	+	+	+	+	+	+	—
Aa 1904(14)	♂	+	+	+	+	+	+	+	+	+	+	—	+	—

+ = fertile crosses. 0 = sterile crosses. — = not crossed.

From the results presented in Table 7 it can be stated, even though certain crosses could not be effected, that the progeny of this cross fall into two genotypic classes of plants. One class containing sibs 3, 6, 7, 10 and 11 were reciprocally sterile with the tester sib 1, and reciprocally fertile with the tester sib 2, while the other class containing sibs 4, 5, 8, 9, 13 and 14 were reciprocally fertile with sib 1 and reciprocally sterile with sib 2.

All the sibs tested were fully fertile when back-crossed as pollen parents to 675(2) 3Ma (2P)—S_4S_4, but completely sterile as ovular parents in the same back-crosses. Full fertility was exhibited by all the reciprocal crosses made with the unrelated parent Aa 1904(14)—S_xS_y.

From these results it is evident that each one of the progeny carried an S_4 factor derived from the homozygous parent, and that in addition six carried an S_x factor, while the remaining seven carried an S_y factor as was expected from the genetic constitution of the parents. It is concluded, therefore, that 675(2) 3Ma (2P) was homozygous for the S factor and that S_4 is a member of the same allelomorphic series as S_x or S_y.

(5) *Identity of the sterility factors S_1, S_2, S_3 and S_4*

In order to determine the relationships of the S factors which have been designated above as S_1, S_2, S_3 and S_4, certain crosses were made between the homozygous genotypes. The results are given in Table 8.

Table 8. *The percentage fertility of crosses involving the homozygotes S_1S_1, S_2S_2, S_3S_3 and S_4S_4*

		Female			
		S_1S_1	S_2S_2	S_3S_3	S_4S_4
Male	S_1S_1 628(2) 2Ma (2P)	⊕	61	79	30
	S_2S_2 393(2) 4Ma (2P)	—	⊕	43	73
	S_3S_3 604(2) 3Ma (1P)	56	70	2	44
	S_4S_4 675(2) 3Ma (1P)	—	61	—	⊕

⊕ = sterile selfings. — = not crossed.

As is seen from Table 8 all the nine cross-pollinations were effective. Crosses between homozygotes bearing the same S factors would normally be ineffective, whereas those between homozygotes bearing different factors would be reciprocally effective. It is

evident, therefore, from these results that the homozygotes tested carried different S factors.

The compatibility reaction of the three progenies resulting from crosses between homozygous individuals was further investigated.

(1) *Progeny resulting from the cross* $S_1S_1 \times S_3S_3$. Eleven sibs from this cross were tested with one sister plant—eight as females only and three as both males and females. As was expected from the constitutions of the parents, all the crosses were sterile. When out-crossed with an unrelated plant the tester sib gave a seed set of 62% as female and 82%, as male parent, which showed it to be normally functional in compatible crosses.

(2) *Progeny resulting from the cross* $S_2S_2 \times S_3S_3$. Thirty-nine plants raised from this cross were isolated in a greenhouse and pollinated by means of sterilized humble bees. In a total of 136 heads examined (an average of 3·5 heads per plant) there was no evidence of seed setting, indicating that all the sibs were inter-sterile, and hence of the same genetic constitution as regards the sterility factors.

(3) *Progeny resulting from the cross* $S_3S_3 \times S_4S_4$. Out of the twelve sibs raised from this cross, eight were crossed as females and three both as males and females with tester sib 6. From the fourteen crosses only one seed was obtained. This result indicates that all the sibs bore like pairs of sterility factors—one derived from the S_3S_3 and the other derived from the S_4S_4 parent.

Sib 6, when out-crossed to an unrelated plant 1447 aA(1) 2, gave 67·4 and 78·6% seed setting when used as male and female parent, respectively.

The above results show that the sterility factors S_1, S_2, S_3 and S_4 were genetically different and that the four were allelomorphs of one series of factors governing sterility in red clover.

V. Self-fertility in red clover—the factor S_f

It has already been shown by Williams & Silow (1933) that the plant 344(1) 263 was self-fertile. This phenomenon of self-fertility has been explained on the assumption that one of the allelomorphs of the S locus has lost its inhibitory potency, and therefore permits the pollen grain carrying the same allele as those carried by the pistil to traverse the style at a rate equal to that of unlike alleles. Williams & Silow further showed that all the progeny of the self-fertile individual 344(1) 263 were self-fertile, and that the progeny of a cross between the self-fertile individuals and unrelated plants belonged to two classes —one self-fertile and the other self-sterile. Unfortunately the parent plants of family 344(1) were not tested for self-fertility, so that their precise constitution is not known. It is, however, concluded, since only one out of twenty-one individuals of family 344(1) was self-fertile, that both of the original parents were self-sterile, and that the self-fertile plant arose as a mutation. It was not determined whether the self-fertile plant 344 aA(1) 263 was homozygous for the self-fertility factor, but certain indirect evidence indicates that the original self-fertility gene originated in a heterozygote.

Self-fertility of L_2 *and* L_3 *progeny from the self-fertile plant* 344(1) 263. In the paper by Williams & Silow referred to, it was shown that the fourteen L_1 sibs obtained as a result of selfing 344(1) 263 were all fully self-fertile. Nine of these fourteen L_1 plants were again selfed, giving L_2 progeny, and two of the L_2 progeny were also selfed, yielding L_3 progeny. Certain individuals from amongst the L_2 and L_3 progeny were tested for self-fertility and the results are set out in Table 9.

Table 9 shows that of 119 L_2 and L_3 progeny plants derived from the original self-fertile plant 344(1) 263, only ten failed to set seed when self-pollinated. Seven of the ten self-sterile plants occurred in L_3, and it is concluded from observations made on the poor quality of the pollen in these sterile individuals that they were cases of functional sterility brought about by inbreeding rather than an inherent incapacity for self-fertility. These results show that the self-fertility factor was regularly transmitted to the progeny over three generations of selfing, and that all the progeny exhibited full fertility apart from such individuals as may have been rendered functionally sterile by inbreeding. Since the self-fertile parent 344(1) 263 is shown below to be heterozygous for self-fertility, it is possible that some homozygous self-sterile plants may have been produced on selfing through pseudo-fertility. This fact may also account for a few of the self-sterile selfings shown in Table 9.

Table 9. *The result of selfing L_2 and L_3 progeny derived from* 344(1) 263

	L_2 and L_3 progeny		
	Total no. of plants selfed	Fertile	Sterile
L_1 parents:			
344(2) 263 Ma (2P) Ma	5	5	0
,, (3P) Ma	6	5	1
,, (7P) Ma	10	10	0
,, (9P) Ma	5	5	0
,, (10P) Ma	3	3	0
,, (11P) Ma	15	14	1
,, (12P) Ma	3	3	0
,, (13P) Ma	2	2	0
,, (14P) Ma	35	34	1
L_2 parents:			
344(1) 263 Ma (11P) Ma (8P) Ma	19	18	1
344(1) 263 Ma (17P) Ma (74P) Ma	16	10	6
Total	119	109	10

(1) *Genetic constitution of* 344(1) 263

Since no direct tests had been made to determine whether the original self-fertile plant was homozygous or heterozygous for self-fertility, seven L_1 plants were mated with entirely unrelated plants, and the progeny tested for self-fertility. Assuming that the plant 344(1) 263 was homozygous for fertility and of the constitution S_fS_f, then all the L_1 plants would also be S_fS_f, and all the progeny obtained from crossing with unrelated plants would be self-fertile. On the other hand, if the original plant was heterozygous for self-fertility—S_fS_a—its L_1 progeny should be S_fS_f and S_fS_a in the ratio of 1 : 1. Crosses between the S_fS_a L_1 progeny and unrelated plants—S_xS_y—would give self-fertile (S_fS_x or S_fS_y) and self-sterile (S_aS_x or S_aS_y) progeny in equal proportions.

The first six L_1 plants reported in Table 10 gave a certain number of both self-sterile and self-fertile plants. The total number of self-fertile and self-sterile plants in these families were in the ratio of 57 : 65—a reasonably close fit to a 1 : 1 ratio. The L_1 plant 344(1) 263 Ma (2P) when crossed with the unrelated plant 626(2) $\frac{(8P)^1}{4\times 5}$ gave twenty progeny plants, all of which were self-fertile.

It is clear, therefore, that the L_1 plants nos. 1, 7, 9, 10, 13 and 14 were heterozygous (S_fS_a) for the self-fertility factor, while the results given by L_1 plant 2 strongly suggest that it was homozygous (S_fS_f) for self-fertility. These results are explicable only if the

original self-fertile plant 344(1) 263 was heterozygous (S_fS_a) for the allelomorphs governing the fertility relationships of red clover plants.

Table 10. *The self-fertility of progeny obtained from crossing L_1 progeny of* 344(1) 263 *with unrelated plants*

L_1 progeny of 344(1) 263	×	Unrelated plants	Total no. of sibs tested	No. of self-fertile sibs	No. of self-sterile sibs	1 : 1 ratio χ^2	1 : 1 ratio P
S_fS_a progeny							
1P	×	626(2) $\frac{8P}{4\times5}$	16	3	13	5·06	0·05–0·02
7P	×	836(1) 7	8	3	5	0·13	0·80–0·70
9P	×	543(1) $\frac{2}{3\times4}$	9	4	5	0·00	1·00
9P	×	632(1) $\frac{25}{1\times6}$					
9P	×	629(1) 5	8	3	5	0·13	0·80–0·70
9P	×	627 $\frac{17P}{2\times3}$	8	4	4	0·00	1·00
10P	×	556(2) $\frac{69\times256}{2P\times3P}$					
13P	×	629(1) $\frac{3}{3\times4}$	12	3	9	2·08	0·20–0·10
13P	×	549(1) 3	10	6	4	0·10	0·80–0·70
13P	×	612(1) 7					
13P	×	820(1) 6	9	5	4	0·00	1·00
13P	×	670(1) 5					
13P	×	556(3) $\frac{56\times162}{2\times3}$ (6P)	10	4	6	0·10	0·80–0·70
13P	×	556(3) $\frac{56\times162}{2\times3}$ (4P)					
14P	×	836(1) 4	12	8	4	0·75	0·50–0·30
14P	×	557(2) $\frac{2}{1\times2}$					
14P	×	1044(1) 4	10	7	3	0·90	0·50–0·30
14P	×	629(3) $\frac{1P}{1\times5}$					
14P	×	565(1) 48	10	7	3	0·90	0·50–0·30
		Total	122	57	65	0·40	0·70–0·50
S_fS_f progeny							
2P	×	626(2) $\frac{8P}{4\times5}$	20	20	0	—	—

Total $\chi^2=10{\cdot}15$; $n=12$; $P=0{\cdot}70$–$0{\cdot}50$.

	χ^2	D.F.	P	Mean square
Deviation	0·40	1	0·70–0·50	—
Heterogeneity	9·75	11	0·70–0·50	0·88
	10·15	12	0·70–0·50	—

(2) *Self-fertility of L_1 progeny obtained from crossing* 344(1) 263 ♂ *with a self-sterile sib* 344(1) 266 ♀ (S_fS_a ♂ × S_aS_x ♀)

The cross between the sibs 344(1) 263 ♂ and 344(1) 266 ♀, which were respectively self-fertile and self-sterile, yielded F_2 progeny, all of which were self-fertile. The self-fertile plant has been shown above to be heterozygous (S_fS_a) for self-fertility, and it is therefore indicated that the self-sterile sib 344(1) 266 contained one factor (S_a) in common with 344(1) 263. Inter-crosses between these two sibs would therefore contain two self-fertile genetic types (S_fS_a and S_fS_x).

The F_2 plants were further selfed and the L_1 progeny so obtained were tested for self-fertility. The results of these tests are set out in Table 11.

Table 11. *Self-fertility of L_1 progeny from the cross* 344(1) 263 ♂ × 266 ♀ ($S_fS_a \times S_aS_x$)

F_2 derivatives of 344(1) 263 × 266 selfed	No. of L_1 sibs tested	No. of self-fertile sibs	No. of self-sterile sibs
11P	3	2	1
17P	23	23	0
18P	5	4	1
19P	1	1	0
20P	11	11	0
22P	2	2	0
23P	6	6	0
26P	11	11	0
29P	2	2	0
30P	1	1	0
107P	9	9	0
110P	8	8	0
144P	8	8	0
158P	8	7	1
Total	98	95	3

Of ninety-eight L_1 progeny obtained, all but three were self-fertile. Although observation on the quality of the pollen in these three sterile selfings did not indicate functional sterility, it is possible that this may partly account for sterility in these three individuals. It must be borne in mind, however, that since the F_2 parents were of the constitution S_fS_x or S_fS_a, an occasional S_aS_a or S_xS_x genotype might have been produced in L_1, which would also partly account for the sterile selfings shown in Table 11.

(3) *Analysis of progeny resulting from crosses between self-fertile (S_fS_a) and self-sterile (S_xS_y) unrelated plants*

In addition to the crosses indicated in Table 10 where six L_1 plants of the genetic constitution S_fS_a were crossed with unrelated plants, S_xS_y, twelve other crosses of $S_fS_a \times S_xS_y$ have been effected. The progeny from each of the twelve crosses were tested for self-fertility and the results are set out in Table 12.

Table 12. *Self-fertility among progeny derived from $S_fS_a \times S_xS_y$*

Family no.	No. of sibs tested	No. of self-fertile sibs	No. of self-sterile sibs	χ^2	P
976(1)	58	28	30	0·02	0·90–0·80
977(1)	17	9	8	0·00	1·00
1344(1)	9	3	6	0·44	0·70–0·50
1537(1)	10	4	6	0·10	0·80–0·70
2100(1) } 2130(1) }	8 } 4 } 12	2 } 1 } 3	6 } 3 } 9	2·08	0·20–0·10
2100(2) 8 × 2	58	28	30	0·02	0·90–0·80
2127(1)	79	44	35	0·81	0·50–0·30
2128(1)	44	19	25	0·57	0·50–0·30
4039(1)	58	32	26	0·43	0·70–0·50
4705(1)	114	64	50	1·48	0·30–0·20
5168(1)	10	5	5	0·00	1·00
Total	469	239	230	0·17	0·70–0·50

Total $\chi^2 = 5{\cdot}95$; $n = 11$; $P = 0{\cdot}90$–$0{\cdot}80$.

	χ^2	D.F.	P	Mean square
Deviation	0·17	1	0·70–0·50	—
Heterogeneity	5·78	10	0·90–0·80	0·57
	5·95	11	0·90–0·80	—

All the twelve families reported in Table 12 consisted of both self-fertile and self-sterile plants, and they all gave a reasonably close fit, as indicated by the values of P, between the actual and expected frequencies on the basis of 1 : 1 segregation.

The genotypic constitutions of the self-sterile plants found in seven of the $S_fS_a \times S_xS_y$ crosses were also determined. It is expected, on the assumption that the genetic constitution of the parents was as set out in Table 12, that the self-sterile plants represent two genotypes S_aS_x and S_aS_y in equal numbers. Separation of the progeny into S_aS_x and S_aS_y was effected by pollinating with two self-sterile inter-fertile tester sibs. These pollinations were made mostly in one direction, using the tester sib as male. Occasionally reciprocal crosses were also performed. The results of these crosses are set out in Tables 13–16.

In analysing the twenty-five self-sterile plants in family 2128(1), sibs 1 and 3 which were inter-fertile, and which have been assigned the genetic constitution S_aS_x and S_aS_y, respectively, were used as tester sibs. Crosses with the self-fertile sib 8 were also made. It will be seen from Table 13 that eleven sibs showed fertility relationships which indicate that they belong to the genotypic class S_aS_x, while the remaining fourteen sibs behaved as members of the S_aS_y class. All the crosses that were made involving the self-fertile sib 8 were fully compatible.

On the assumption that S_f is a member of the same allelic series as S_a, S_x and S_y, the expected ratio of the possible genotypes resulting from $S_fS_a \times S_xS_y$ would be 2 $S_fS_x + S_fS_y$ (self-fertile) : 1 S_aS_x (self-sterile) : 1 S_aS_y (self-sterile). The ratio observed in family 2128(1), viz. 19 $S_fS_x + S_fS_y$: 11 S_aS_x : 14 S_aS_y agrees reasonably closely ($\chi^2 = 1{\cdot}23$, $P = 0{\cdot}70$–$0{\cdot}50$) with the expected ratio.

Table 14 shows the fertility relationships of forty-five self-sterile plants of family 4075(1). The tester sibs used were nos. 20, 50 and 78 (S_aS_x), and 16, 17, 23 and 42 (S_aS_y). These two groups of sibs were intra-sterile but inter-fertile.

It will be noted that nineteen sibs in Table 14 when inter-crossed with tester sibs 20, 50 or 78 (S_aS_x) were sterile and therefore of the genetic constitution S_aS_x. Unfortunately sibs 62 and 71 were not crossed with any of the sibs of the S_aS_x group, but from their fertility relationship with sibs from the S_aS_y group it is concluded that these two sibs also belonged to the S_aS_x group. All the twenty-one sibs when crossed with sibs 16, 17, 23 or 42 were fully fertile. The remaining twenty-four sibs were fully compatible with the sibs of the S_aS_x group, and hence it is concluded that their genetic constitution was S_aS_y. Twenty-one of these twenty-four sibs when crossed with sibs of the S_aS_y group were entirely sterile.

It will be seen from Table 12 that the total number of self-incompatible sibs in family 4075(1) was 50, but of these only forty-five were analysed. The ratios as far as the above analyses are concerned are set out below:

Family 4075(1)

	Self-fertile class	Self-sterile class		
	S_fS_x or S_fS_y	S_aS_x	S_aS_y	S_aS_x or S_aS_y undetermined
Observed	64	21	24	5
Expected	54·5	27·25	27·25	—

$\chi^2 = 3{\cdot}47$; $P = 0{\cdot}20$–$0{\cdot}10$.

Table 13. *Analysis into the genotypic classes S_aS_x and S_aS_y of twenty-five self-sterile plants of family* 2128(1)

		Self-sterile plants																								
		S_aS_x											S_aS_y													
	Sib nos. ...	1	2	5	7	9	14	16	25	27	33	40	3	4	6	10	12	15	20	21	26	29	34	35	39	43
Tester sibs	No. 1 (S_aS_x)	—	0	0	0	0	0	—	0	⊕	0	0	+	+	+	+	+	+	+	+	—	+	+	+	+	+
	No. 3 (S_aS_y)	+	+	+	+	+	+	+	+	+	+	+	—	0	0	0	0	0	0	0	0	0	0	0	0	0
	No. 8 (S_yS_x)	+	+	+	+	—	—	+	—	—	+	—	+	—	+	+	—	+	+	—	—	+	+	+	—	+

— = not crossed. ⊕ = slight pseudo-fertility. + = fertile crosses. $S_aS_x = 11$; $S_aS_y = 14$.

Table 14. *The analysis into the genotypic classes (S_aS_x and S_aS_y) of forty-five self-sterile progeny of family* 4075(1)

		S_aS_x																				
	Sib nos. ...	3	4	14	20	26	29	43	48	50	54	62	63	68	71	72	74	78	80	90	91	111
Tester sibs	20, 50, 78, 16, 17 S_aS_x	0	0	0	0	0	0	0	0	0	0	—	0	0	—	0	0	0	0	0	0	0
	23, 42 S_aS_y	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

		S_aS_y																								
	Sib nos. ...	16	17	19	23	25	35	36	42	45	47	55	56	59	64	65	70	73	76	79	82	83	84	102	103
Tester sibs	20, 50, 78, 16, 17 S_aS_x	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	23, 42 S_aS_y	0	0	0	0	0	0	—	0	0	—	0	0	0	—	—	0	0	0	0	0	0	0	0	0

— = not crossed.

effected since the publication of that paper in 1931 have fully substantiated the statement, and of the very small proportion of crosses which did prove cross-sterile, the majority were directly attributable to pathological disorders. The fact that the vast majority of non-pedigree red clover plants are cross-fertile suggests the existence of an extensive series of allelomorphic factors governing fertilization in the species.

Various investigators have already reported on the numbers of sterility allelomorphs involved in the following species: *Nicotiana Sanderae et alata*, *Prunus avium*, *Antirrhinum glutinosum*, *Oenothera organensis*, *Trifolium repens* and *T. pratense*. East & Yarnell (1929) reported the isolation of sixteen allelomorphs of the S factor—fifteen sterility alleles $S_1, S_2, \ldots, S_{15}$, and one self-fertility allele S_f—from material selected from *Nicotiana Sanderae* and *N. alata grandiflora*, whilst Crane & Brown (1937) have described cross-pollinations in *Prunus avium* which are interpreted on the basis of a minimum of nine different S alleles. Gruber (1932) claims to have isolated twenty-eight S alleles in a variety of *Antirrhinum glutinosum*, but it may be pointed out that the data on which his conclusions were based demonstrate the occurrence of no more than eight factors. In a natural population of *Oenothera organensis* estimated at about 500 plants, Emerson (1939) reported the presence of thirty-seven different alleles. In an investigation with two natural populations of *Trifolium repens*, Atwood (1944) reported that 73 and 80%, respectively, of the alleles contained in the two samples were different.

II. Object, material and methods

In a paper by Williams & Silow (1933) it was reported that twenty-one red clover plants selected from the Montgomery late-flowering strain were fully cross-fertile in all possible combinations. From this it was concluded on the oppositional factor hypothesis that there were at least seven different sterility allelomorphs operating in this material.

The investigation outlined below was undertaken with the object of determining the number of different sterility allelomorphs in a given number of non-pedigree plants selected at random from commercial populations of red clover. The tests were begun in 1932, and the first analysis was made on twenty-five plants from a population known as English late-flowering red clover. This was followed by a second analysis of twenty plants from another population commonly known as English broad red clover.

The non-pedigree plants used in these tests were each crossed with a plant known to be homozygous for the sterility factors (see this *Journal*, p. 54). The progenies obtained from these crosses were classified by means of appropriate sib tests into their respective genotypes. All the F_1 plants obtained as a result of these crosses would be expected to possess one common sterility factor derived from the homozygous parent as well as an unknown factor derived from the non-pedigree parent. In order to ascertain that the constitution of these progeny genotypes agreed with expectation they were back-crossed with the homozygous parent. All the genotypes were finally cross-pollinated in all possible combinations in order to test the identity of the unknown factor derived from the non-pedigree parent.

Where it was necessary to perform a large number of crosses over several seasons involving one particular plant, clones of the material were prepared and renewed each autumn to ensure an adequate supply of flowers for crossing. The plants were pollinated by hand without previous emasculation, and a minimum of fifty-five and an average sixty-five florets were pollinated in each test. All the pollinations were effected in glass

houses which were protected against contamination by insect pollinators. The sterile combinations were retested several times during two, and sometimes three, successive seasons.

The seeds were threshed by hand and classified as good, fair, and poor, representing, respectively, plump and well developed, under-developed and shrivelled, and very small non-viable seeds.

III. THE NUMBER OF S ALLELES IN TWENTY-FIVE ENGLISH LATE-FLOWERING RED CLOVER PLANTS

It has been previously stated that these twenty-five plants were chosen at random from a variety commonly known as English late-flowering red clover. Experience on crossing red clover plants has shown that plants selected at random from large populations prove to be reciprocally fully fertile, and it is concluded on the oppositional factor hypothesis that such individuals all possess different sterility allelomorphs. It is therefore tentatively assumed that the above twenty-five plants each possessed a different pair of S factors, and they have been designated by the genetic constitution S_xS_y—x and y ranging from 1 to 10.

$$(1)\ S_xS_y \times S_3S_3$$

Each of the twenty-five plants (Aa 2100(1) to Aa 2100(25)) was used as pistillate parent with 604(2) 3Ma (1P). This particular plant has been shown (see p. 57 of this *Journal*) to be of the genetic constitution S_3S_3. The results of these crosses are set out in Table 1.

Table 1. *Percentage seed setting obtained by crossing twenty-five unrelated red clover plants Aa* 2100(1) *to* (25) ♀ *by* 604(2) 3*Ma* (1*P*) ♂—S_3S_3

♀ parents	Florets pollinated	Seed set: Good	Seed set: Fair	Seed set: Total	Percentage set
Aa 2100(1)	75	43	1	44	58·6
,, (2)	47	12	0	12	25·5
,, (3)	70	54	1	55	78·6
,, (4)	53	30	3	33	62·3
,, (5)	51	33	1	34	66·7
,, (6)	62	72	0	72	116·1
,, (7)	72	42	0	42	58·3
,, (8)	108	41	1	42	38·8
,, (9)	73	67	0	67	91·8
,, (10)	71	63	2	65	91·5
,, (11)	71	55	0	55	77·5
,, (12)	112	101	2	103	92·0
,, (13)	56	48	0	48	85·7
,, (14)	74	67	3	70	94·6
,, (15)	87	77	3	80	92·0
,, (16)	54	44	0	44	81·5
,, (17)	99	0	10	10	10·1
,, (18)	62	44	0	44	71·0
,, (19)	56	54	4	58	103·6
,, (20)	61	43	0	43	70·5
,, (21)	59	45	6	51	86·4
,, (22)	58	51	0	51	87·9
,, (23)	69	31	0	31	45·0
,, (24)	63	16	8	24	38·1
,, (25)	71	32	0	32	45·1

It will be noted that the seed set in twenty-four of the above crosses ranges from 26 to 116%, demonstrating that S_3 pollen was fully functional on the styles of twenty-four

out of twenty-five of the female parents. Plant Aa 2100(17) produced only ten poorly developed seeds which failed to germinate, and this might be interpreted as indicating that Aa 2100(17) carried an S_3 factor. Owing to the fact that no further investigations were carried out on this plant, no definite pronouncement can be made regarding its sterility constitution.

The cross-fertility shown in these crosses is interesting, as it gives an indication of the frequency of the S_3 gene. Assuming the possibility of Aa 2100(17) being heterozygous for S_3, it is clear that of the fifty sterility allelomorphs present in the twenty-five female parents utilized in the above crosses, S_3 occurred only once. The forty-eight alleles present in the female parents of the twenty-four fertile crosses possessed no inhibitory action on pollen from 604(2) 3Ma (1P), and it is, therefore, concluded that these were all distinct from the S allele which has been designated as S_3. The frequency of the S_3 gene cannot be regarded as exceptional, as the data presented below demonstrate that the other S factors in red clover possess equally low frequencies.

(2) *Classification of F_1 families into S_3S_x and S_3S_y genotypes*

Since the male parent (604(2) 3Ma (1P)) was homozygous for the sterility factors and of the genetic constitution S_3S_3, the F_1 progeny of each of the twenty-four fertile crosses would be expected to consist of two sterility genotypes, S_3S_x and S_3S_y, in the ratio of 1 : 1. For the purpose of ascertaining the presence of these groups, six plants were tested from each of twenty-two F_1 families, while seventy and eighty-nine plants, respectively, were subjected to tests in the remaining two F_1 families. These tests were effected by means of tester sibs, and the plants were classified as S_3S_x or S_3S_y on the basis of their fertility relationships with the testers. As a check on the constitution of the F_1 plants two inter-fertile (1 S_3S_x and 1 S_3S_y) tester sibs were invariably used for testing each family, and in all cases the results obtained by the initial pollinations with tester sib 1 were corroborated by the results obtained with tester sib 2. The sister plants which were sterile with no. 1 tester sib were fertile with no. 2 tester sib, and vice versa.

The results of these tests are set out in Tables 2 and 3.

Table 2. *The number of plants belonging to the S_3S_x and S_3S_y genotypes in each of the twenty-four F_1 families*

Family no.	No. of S_3S_x plants	No. of S_3S_y plants	Family no.	No. of S_3S_x plants	No. of S_3S_y plants
1	3	3	13	2	4
2	2	4	14	2	4
3	2	4	15	3	3
4	4	2	16	5	1
5	3	3	18	3	3
6	42	28	19	1	5
7	5	1	20	3	3
8	4	2	21	4	2
9	5	1	22	1	5
10	4	2	23	5	1
11	2	4	24	3	2
12	44	45	25	2	4

Total = 154 S_3S_x : 136 S_3S_y. ($\chi^2 = 1{\cdot}12$; $P = 0{\cdot}30$–$0{\cdot}20$.)

No difficulty was experienced in distinguishing between compatible and incompatible crosses in these tests, and from Table 3 it will be noted that the compatible crosses gave an average seed set of 77·9%. Of the 120 incompatible crosses, on the other hand, 11

yielded a seed set of from 0 to 5%, the other five crosses yielding between 5 and 10%. The average seed set for all the incompatible matings was 0·84%, being the result of pseudo-fertilization.

Table 3. *Frequency table showing fertility of the crosses depicted in Table 2*

	Percentage fertility. Class limits													
	0	5	10	20	30	40	50	60	70	80	90	100	100+
No. of crosses	115	5			1	2	5	14	22	29	39	21	13
	Sterile				Fertile								

Average percentage seed set in sterile crosses = 0·84. Average percentage seed set in fertile crosses = 77·9.

Table 2 demonstrates that each of the twenty-four F_1 families consisted of two inter-fertile, intra-sterile genetic groups, S_3S_x and S_3S_y. The total distribution of 154 S_3S_x : 136 S_3S_y agrees closely with expectation on the basis of a 1 : 1 ratio. F_1 family 12, of which eighty-nine plants were tested, gave a perfect fit with a 1 : 1 ratio, while family 6, of which seventy plants were tested, although not exhibiting such a close agreement with equality as the former family, cannot be regarded as deviating significantly from expectation. The calculated value of χ^2 for the segregation in family 6 is 2·80 and P is 0·10–0·05.

(3) *Back-crossing S_3S_x and S_3S_y genotypes to the S_3S_3 parent*

One plant of each of the forty-eight genotypes (24 S_3S_x and 24 S_3S_y) was isolated on the basis of the tests described in the preceding section, and reciprocally back-crossed to the S_3S_3 parent, 604(2) 3Ma (1P). As an additional check on the constitution of these forty-eight plants each was tested for self-fertility. On the basis of the constitution of the parents used in these crosses ($S_xS_y \times S_3S_3$), each F_1 plant should have possessed an S_3 factor, and hence would be expected to be fertile as male, but sterile as female in back-crosses involving the homozygous S_3S_3 parent.

The results obtained from selfing and back-crossing are shown in Table 4.

Table 4 shows that all the plants used in these tests were cross-fertile as male but cross-sterile as female with the S_3S_3 parent. The results demonstrate the presence of an S_3 factor as well as an S_x or S_y factor in all the F_1 progeny plants tested. Unfortunately, a few crosses were inadvertently omitted, but the results are sufficiently complete to conclude that each of these F_1 progeny plants contained one unknown S factor—the other being an S_3 factor derived from 604(2) 3Ma (1P).

(4) *Tests of identity of forty-eight S_x and S_y alleles*

In order to determine the actual number of similar alleles carried by the twenty-four original English late-flowering red clover plants which yielded F_1 families, representative plants from each of forty-eight S_3S_x or S_3S_y genotypes were inter-crossed in all possible combinations. For the purpose of these tests one sister plant was selected from each of the forty-eight genotypic classes. All the crosses which were obviously fertile were performed in only one direction, while those which gave a seed set of less than 30% were repeated in both directions. All the incompatible combinations were repeated twice or three times in different seasons. The results of these tests are set out in Tables 5–7.

It will be seen from the results presented in Table 6 that the differences in percentage seed set between the fertile and sterile combinations were quite clearly defined. The percentage seed set in the sterile crosses ranged from 0 to 6·3, while in the fertile crosses

Table 4. *The percentage fertility obtained from selfing and back-crossing one S_3S_x and one S_3S_y plant from each of the twenty-four F_1 families*

(*Note.* 1*x* and 1*y* in Table 4, and all subsequent tables, represent S_3S_x and S_3S_y plants from F_1 family 1, while 2*x* and 2*y* represent S_3S_x and S_3S_y plants in family 2, etc.)

S_3S_x and S_3S_y plant no.	Selfing			Back-crossing on to 604(2) 3Ma (1P)—S_3S_3					
				S_3S_3 ♂			S_3S_3 ♀		
	No. of florets	No. of seeds	Percentage set	No. of florets	No. of seeds	Percentage set	No. of florets	No. of seeds	Percentage set
1*x*	43	2	4·7	37	0	0	43	17	39·5
1*y*	57	0	0	50	0	0	50	36	72·0
2*x*	61	2	3·3	52	0	0	52	28	53·8
2*y*	76	1	1·3	50	0	0	49	30	61·2
3*x*	53	0	0	39	2	5·1	59	33	55·9
3*y*	58	3	5·2	36	0	0	44	35	79·5
4*x*	52	0	0	45	0	0	50	15	30·0
4*y*	65	0	0	46	0	0	49	23	46·9
5*x*	61	0	0	59	2	3·4	33	18	54·5
5*y*	58	0	0	55	0	0	54	27	50·0
6*x*	59	0	0	52	0	0	56	27	48·2
6*y*	59	0	0	41	0	0	62	35	56 4
7*x*	49	0	0	41	0	0	50	43	86·0
7*y*	75	0	0	62	1	1·6	44	26	59·1
8*x*	57	0	0	40	0	0	45	13	28·9
8*y*	37	0	0	37	2	5·4	42	21	50·0
9*x*	67	2	3·0	50	0	0	45	38	84·4
9*y*	62	0	0	45	1	2·2	44	34	77·3
10*x*	54	0	0	49	3	6·1	55	35	63·6
10*y*	55	0	0	52	2	3·8	51	56	109·8
11*x*	38	0	0	50	0	0	46	36	78·3
11*y*	41	1	2·4	37	4	10·9	55	39	70·9
12*x*	45	2	4·4	46	4	8·7	50	49	98·0
12*y*	43	0	0	35	2	5·7	35	14	40·0
13*x*	51	5	9·8	32	3	9·4	60	36	60·0
13*y*	72	1	1·4		Not tested		36	31	86·1
14*x*	63	0	0	72	1	1·4	44	33	75·0
14*y*	57	0	0		Not tested		49	28	57·1
1[illegible]	54	1	1·8		Not tested		33	25	75·7
15*y*	56	0	0	62	4	6·4	85	25	29·4
16*x*	[illegible]	0	0	58	0	0	53	45	84·9
16*y*	79	0	0	54	0	0	36	41	113·9
18*x*	55	2	3·6	61	0	0	55	26	47·3
18*y*	33	0	0	50	0	0	53	22	41·5
19*x*	37	0	0	52	1	1·9	51	37	72·5
19*y*	51	1	1·9	49	0	0	49	39	79·6
20*x*	57	0	0	36	0	0	47	48	102·1
20*y*	73	0	0	90	0	0	43	40	93·0
21*x*	84	0	0	90	0	0	41	36	87·8
21*y*	69	0	0	39	0	0	45	29	64·4
22*x*	80	0	0	75	0	0	51	41	80·4
22*y*	41	0	0	46	0	0	51	26	50·9
23*x*	46	0	0	31	0	0	37	18	48·7
23*y*	74	0	0	32	0	0	44	25	56·8
24*x*	94	1	1·1	50	3	6	46	43	93·5
24*y*	51	0	0	40	2	5	36	35	97·2
25*x*	62	0	0	42	0	0	35	28	80·0
25*y*	66	0	0		Not tested			Not tested	

the range extended from 20·7 to 172·5. Though these results indicate that certain plants were more highly cross-fertile than others in compatible pollinations, it is probable that external conditions were in the main largely responsible for the differences in percentage

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Table 5. *The percentage fertility obtained in diallel crosses involving one plant out of each of the forty-eight sterility genotypes obtained from twenty-four F_1 families* ($S_xS_y \times S_3S_8$)

	1x	1y	2x	2y	3x	3y	4x	4y	5x	5y	6x	6y	7x	7y	8x	8y	9x	9y	10x	10y	11x	11y	12x	12y	13x	13y	14x	14y	15x	15y	16x	16y	18x	18y	19x	19y	20x	20y	21x	21y	22x	22y	23x	23y	24x	24y	25x	25y
1x	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1y	85	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2x	76	73	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2y	37	98	95	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3x	115	94	68	65	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3y	96	64	73	62	77	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4x	113	101	74	96	77	74	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4y	98	100	90	100	66	86	72	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5x	96	100	75	70	89	97	106	60	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5y	57	100	96	85	71	54	103	77	66	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6x	92	102	81	77	83	94	101	87	65	104	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6y	21	101	80	71	81	122	95	74	81	106	57	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7x	28	73	85	71	1	73	59	72	63	110	83	64	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7y	83	112	69	97	55	91	85	98	74	85	75	80	85	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8x	36	95	89	51	86	89	87	90	100	100	75	33	71	75	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8y	100	91	77	92	82	95	83	102	84	102	103	78	76	81	107	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9x	47	85	93	112	93	96	92	97	87	122	79	83	83	63	95	91	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9y	63	100	159	106	82	1	106	119	105	121	110	94	98	88	104	98	98	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10x	83	96	70	67	88	97	72	93	98	90	102	93	90	78	99	85	61	115	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10y	52	96	84	62	83	97	108	98	98	92	74	105	96	94	83	75	109	122	86	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
11x	100	90	110	102	84	84	100	90	73	65	106	101	59	78	112	77	77	145	98	113	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
11y	75	84	111	73	102	98	102	123	100	110	85	114	71	96	81	65	62	132	69	115	50	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12x	57	80	118	63	98	107	102	103	116	81	108	88	95	70	90	92	71	95	55	114	48	85	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12y	68	103	113	88	80	89	98	100	100	108	112	72	88	60	122	76	48	135	57	42	98	115	116	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
13x	6*	98	122	87	78	63	103	90	92	109	98	83	83	64	134	56	88	117	90	97	86	90	86	60	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
13y	39	104	85	91	81	91	83	97	96	93	98	85	114	54	107	89	84	117	38	100	64	103	86	67	123	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
14x	40	72	132	92	103	95	98	103	92	126	83	108	80	57	125	93	91	115	82	95	51	94	59	108	103	81	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
14y	102	98	92	115	88	89	78	124	98	84	80	81	105	50	113	103	65	83	91	103	75	86	91	93	133	107	70	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
15x	119	94	78	119	81	103	132	105	119	131	100	91	83	86	113	92	70	110	95	108	70	90	83	98	91	77	87	92	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
15y	50	100	109	76	100	85	114	107	84	117	103	93	95	73	97	93	79	80	86	97	83	76	44	94	83	68	75	108	86	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
16x	127	118	91	143	96	96	93	102	96	75	90	102	98	53	95	94	105	149	86	131	45	111	82	94	143	93	121	93	66	115	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
16y	95	117	109	104	78	93	111	107	97	88	125	73	118	47	114	93	108	115	100	102	71	66	60	59	143	104	47	77	104	60	96	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
18x	98	44	74	100	96	71	71	100	88	134	105	89	98	78	109	82	66	109	111	102	87	149	1	107	105	83	93	84	90	99	87	149	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
18y	123	95	98	93	94	88	93	84	94	95	97	78	111	2	125	87	103	110	65	117	77	85	90	102	96	91	99	127	97	88	94	119	61	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
19x	116	120	76	102	87	90	96	89	107	105	95	81	98	60	86	98	115	115	90	87	61	139	105	103	89	88	92	95	87	90	67	103	68	102	—	—	—	—	—	—	—	—	—	—	—	—	—	—
19y	90	93	103	94	110	111	107	97	92	132	154	47	100	40	121	86	104	107	94	92	68	90	143	108	111	102	86	100	90	127	99	108	105	88	84	—	—	—	—	—	—	—	—	—	—	—	—	—
20x	101	107	124	81	94	93	0	92	105	99	91	78	96	100	90	82	100	109	27	104	69	121	78	127	95	73	88	116	86	97	88	112	66	99	91	53	—	—	—	—	—	—	—	—	—	—	—	—
20y	94	93	116	100	88	94	107	61	73	110	133	78	106	77	105	32	103	78	102	128	75	89	94	100	113	94	100	107	88	95	86	113	96	53	104	66	94	—	—	—	—	—	—	—	—	—	—	—
21x	76	65	120	91	86	69	97	37	90	110	102	45	81	79	89	84	108	58	43	104	66	91	86	97	100	91	96	50	50	97	103	150	102	91	116	93	114	54	—	—	—	—	—	—	—	—	—	—
21y	63	108	89	72	86	98	70	29	84	86	149	177	89	41	115	82	100	82	110	117	70	139	104	72	100	91	95	97	108	114	90	125	99	86	67	83	86	98	57	—	—	—	—	—	—	—	—	—
22x	70	93	116	82	69	95	67	78	93	104	56	49	120	55	105	63	58	121	94	89	88	83	70	96	118	105	115	53	85	69	107	124	86	122	79	58	67	86	71	105	—	—	—	—	—	—	—	—
22y	96	93	98	107	89	36	82	83	84	95	96	106	83	103	110	78	108	98	112	106	93	78	57	92	83	109	109	114	80	89	81	92	87	127	109	93	102	73	117	96	96	—	—	—	—	—	—	—
23x	110	88	111	124	80	98	99	72	98	110	86	88	112	114	106	62	77	90	86	94	77	112	115	102	82	79	108	30	70	126	67	143	50	91	117	66	86	77	67	98	100	80	—	—	—	—	—	—
23y	77	88	104	116	63	92	48	47	78	115	78	96	103	57	117	95	106	93	61	130	88	97	90	92	85	69	87	88	80	96	85	89	39	74	36	99	76	100	81	74	80	63	45	—	—	—	—	—
24x	72	104	132	92	76	100	61	125	88	102	96	106	97	89	98	57	110	96	41	96	83	117	109	50	109	100	95	57	57	73	74	142	92	123	83	69	89	73	60	83	133	67	51	92	—	—	—	—
24y	73	84	173	81	78	100	60	122	103	129	93	109	109	95	94	98	75	77	95	71	57	84	125	104	119	83	79	173	73	83	104	118	68	86	97	84	56	72	108	109	98	105	85	95	43	—	—	—
25x	28	100	81	104	77	105	63	109	93	100	56	108	90	84	98	92	117	102	78	81	94	107	90	112	102	75	107	49	82	85	106	130	82	98	62	92	86	82	62	84	40	73	46	96	94	89	—	—
25y	101	100	105	69	80	96	69	100	83	123	53	100	100	110	95	73	84	102	39	87	61	53	78	90	119	87	71	56	86	84	91	117	74	84	80	93	87	1	81	99	95	86	53	88	35	73	73	—

* Figures in black type denote incompatible matings

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fertility exhibited by the fertile combinations. As stated, all fertile crosses yielding a seed set of less than 30% were repeated in both directions, and the results from these repeat crosses were invariably very different from the first cross. Instances were met with where crosses which yielded a seed set of less than 20% in one season gave over 80% in the second season. As will be seen from Table 6, only five crosses of the 1121 fertile crosses did not exceed the 30% seed-set mark, and even these were quite easily differentiated from the sterile combinations which were fully sterile or only very slightly pseudo-fertile.

Table 6. *Frequency table showing fertility of the crosses depicted in Table* 5

	Percentage fertility. Class limits																
	0	2	4	6	10	20	30	40	50	60	70	80	90	100	100+
No. of crosses	6			1			5	13	24	49	76	126	211	259	358
	Sterile						Fertile								

Table 7. *The percentage fertility of the seven incompatible cross-pollinations shown in Table* 5

Cross	♀ plant in cross	No. of florets pollinated	Seed set	Percentage set
$1x \times 13x$	$1x$	552	12	2·2
	$13x$	528	56	10·6
$3x \times 7x$	$3x$	369	0	0·0
	$7x$	345	9	2·6
$3y \times 9y$	$3y$	378	6	1·6
	$9y$	297	4	1·3
$4x \times 20x$	$4x$	349	0	0·0
	$20x$	285	0	0·0
$7y \times 18y$	$7y$	224	7	3·1
	$18y$	301	6	1·9
$12x \times 18x$	$12x$	168	4	2·4
	$18x$	186	1	0·5
$25y \times 20y$	$25y$	160	0	0·0
	$20y$	279	3	1·1

Table 5 shows the percentage fertility obtained from the 1128 crosses involved in these tests. From these results it will be noted that seven of the crosses were sterile or only slightly pseudo-fertile, and the details of the pollinations performed in these crosses are summarized in Table 7. Since each genotype carried only one unknown S_x or S_y allelomorph, plants in which the unknown alleles were similar should be cross-sterile, while all those bearing dissimilar S_x or S_y alleles should be cross-fertile. On the basis of the fertility relationships detailed above, it is concluded that all the S factors involved were different, with the exception of the following:

S_x plant 1 = S_x plant 13
S_x ,, 3 = S_x ,, 7
S_y ,, 3 = S_y ,, 9
S_x ,, 4 = S_x ,, 20
S_y ,, 7 = S_y ,, 18
S_x ,, 12 = S_x ,, 18
S_y ,, 20 = S_y ,, 25

It is thus evident that of the forty-eight sterility allelomorphs carried by the original twenty-four English late-flowering red clover plants, forty-one were different—thirty-four being represented only once and seven represented twice.

IV. The number of S alleles in twenty English broad red clover plants

This investigation on a random selection of twenty plants of English broad red clover was undertaken to confirm the surprising results obtained with the twenty-five English late-flowering red clover plants. Since these twenty plants were presumably unrelated, it was tentatively assumed that they all possessed different S alleles.

(1) $S_xS_y \times S_1S_1$

As a first step in determining the number of different S alleles carried by the twenty English broad red clover plants, each individual was crossed with the homozygous S_1S_1 plant,* 628(2) 2Ma (2P). The results of seed setting in these crosses are set out in Table 8.

Table 8. *The results of crossing twenty unrelated plants of English broad red clover, S_xS_y ♂, with* 628(2) 2Ma (2P), S_1S_1 ♀

Plant no.	Florets pollinated	Seed set	Percentage set	Plant no.	Florets pollinated	Seed set	Percentage set
1	58	47	81·0	11	46	22	47·8
2	53	16	30·2	12	47	36	76·6
3	71	24	33·8	13	35	6	17·1
4	59	22	37·3	14	16	9	56·2
5	54	22	40·7	15	52	29	55·8
6	43	11	25·6	16	38	26	68·5
7	41	14	34·1	17	33	26	78·8
8	46	6	13·0	18	35	20	57·1
9	53	51	96·2	19	54	22	40·7
10	40	26	65·0	20	38	22	57·9

It will be noted from Table 8 that all the crosses with the exception of nos. 8 and 13 gave a percentage seed set ranging from 25·6 to 96·2. Nos. 8 and 13 gave a seed set of only 13·0 and 17·1 %, respectively, but while this must be regarded as a low seed set in fertile crosses of red clover, the number of florets pollinated (forty-six in no. 8 and thirty-five in no. 13) was not sufficient to enable one to draw any conclusion as to the precise fertility relationships of these plants with 628(2) 2Ma (2P).

(2) *Classification of F_1 families into S_1S_x and S_1S_y genotypes*

Unfortunately it was possible to raise only one F_1 plant from each of families 13 and 17. Twenty plants from family 1 and four to six plants from the remaining seventeen F_1 families were selected and classified on the basis of their fertility reaction with tester sibs into S_1S_x or S_1S_y classes. The number of tester sibs used in these tests varied from one to three in different families. All plants which were sterile with no. 1 tester and fertile with no. 2 tester have been assigned the constitution S_1S_x, while all those which were fertile with no. 1 tester and sterile with no. 2 tester were designated as S_1S_y. The distinction between fertile and sterile crosses was in all cases quite definite—the average seed setting being 60·4 % for the fertile combinations and 0·8 % for the sterile combinations.

The results of these tests are set out in Table 9.

On reference to Table 9 it will be seen that each of the eighteen F_1 progenies consisted of two approximately equal-sized genotypic groups S_1S_x and S_1S_y, as was expected from the genetic constitution of the parent plants.

* For details of the genetic constitution of 628(2) 2Ma (2P), see this *Journal*, p. 54.

(3) *Back-crossing S_1S_x and S_1S_y genotypes to the S_1S_1 parent*

In order to confirm the presence of the S_1 factor in the progeny derived from the $S_xS_y \times S_1S_1$ crosses, plants selected from twenty-four S_1S_x and S_1S_y genotypic groups were back-crossed both as male and female to the homozygous S_1S_1 parent. Before attempting the analysis of identity of the S_x and S_y alleles it was further considered advisable to test each of the plants involved in the inter-group crosses shown in Table 12 for self-fertility. The results of back-crossing and selfing are shown in Tables 10 and 11, respectively.

Table 9. *Classification of F_1 progeny (S_1S_x or S_1S_y) resulting from crossing eighteen unrelated plants (S_xS_y) with a homozygous S_1S_1 plant*

F_1 family no.	No. of tester sibs used	No. of S_1S_x genotypes	No. of S_1S_y genotypes
1	1 and 3	12	8
2	1	3	2
3	1 and 2	2	3
4	1, 3 and 4	3	2
5	1	1	4
6	5 and 6	4	1
7	1 and 2	3	2
8	1 and 2	2	3
9	2	2	3
10	1	1	4
11	6	3	2
12	5 and 6	2	3
14	3 and 4	1	3
15	1, 2 and 3	5	1
16	1 and 2	3	2
18	5 and 6	3	2
19	1	1	4
20	4 and 6	2	3

Total: 53 S_1S_x : 52 S_1S_y.

Note. F_1 families 13 and 17 from which only one plant was raised have been omitted from the above table.

Table 10. *Results of back-crossing to the homozygous parent (S_1S_1) of representative plants of twenty-four genotypes derived from the $S_xS_y \times S_1S_1$ crosses*

	No. of genotypes											
Direction of cross ...	1x	1y	2x	2y	3x	6y	7x	7y	8y	9x	10x	11x
S_1S_x or S_1S_y ♀ × S_1S_1 ♂	0	0	0	0	⊕	0	0	0	0	0	0	0
S_1S_1 ♀ × S_1S_x or S_1S_y ♂	–	+	+	+	+	+	+	+	–	+	+	+
Direction of cross ...	11y	12x	12y	13x	14x	15x	15y	16x	16y	17x	18x	20x
S_1S_x or S_1S_y ♀ × S_1S_1 ♂	0	⊕	0	–	0	0	0	0	0	0	0	0
S_1S_1 ♀ × S_1S_x or S_1S_y ♂	+	+	–	+	+	–	+	–	+	+	–	+

0 = full sterility. + = full fertility. ⊕ = pseudo-fertility. – = not crossed.

Table 11. *Frequency table showing percentage pseudo-fertility of S_1S_x and S_1S_y plants derived from the crosses $S_xS_y \times S_1S_1$*

	Percentage fertility. Class limits							
	0	0–1	1–2	2–3	3–4	4–5	5–6	6–7
No. of plants	93	0	0	3	1	2	0	4
	7–8	8–9	9–10	10–12	12–14	14–16	16–18	
No. of plants	0	0	1	0	1	1	0	

Total no. of plants = 106.

As may be seen from Table 10, all the back-crosses where the homozygous S_1S_1 plant was used as male parent were sterile with the exception of two plants—3x and 12x—which exhibited pseudo-fertility to the extent of 5·0 and 3·3%, respectively. When the

crosses were performed in the opposite direction, full fertility resulted, the average seed setting for the fertile pollinations being 36·47 % of florets pollinated. Although the results presented in Table 10 are not quite complete, they serve to demonstrate the inhibitory action conditioned by the S_1 allele in the S_1S_x and S_1S_y plants when pollinated by S_1 pollen from the homozygous parent. In the crosses S_1S_1 ♀ × S_1S_x or S_1S_y ♂, the S_x or S_y allele promoted full fertility.

From the results of selfing 106 S_1S_x and S_1S_y plants set out in Table 11 it is seen that ninety-three plants produced no seed, while ten of the pseudo-fertile plants gave a seed set of less than 7 %. The remaining three pseudo-fertile plants yielded from 9 to 15 % seed. Although the latter exhibited rather a high percentage of self-fertilization for red clover, there is no reason to doubt that they were genuine instances of pseudo-fertility.

(4) *Tests of identity of forty S_x and S_y alleles*

The thirty-eight S_x and S_y alleles (one S_x and one S_y from each of eighteen families and one S_x from each of two families) which had been isolated from F_1 families derived from crossing twenty English broad red clover plants with S_1S_1 were inter-pollinated with each other in all possible combinations. In addition, two other genotypes $21x$ and $22x$ were included in this series. Plant $21x$ was a sister plant of the homozygous S_1S_1 parent used in these crosses and had been previously shown to be heterozygous for the S_1 factor, while $22x$ was a plant selected from a family obtained by crossing the homozygous S_1S_1 plant with another homozygous S_2S_2 plant. The genetic constitution of $22x$ was therefore S_1S_2.

As in the case of the diallel crosses performed on the English late-flowering derivatives, most of the pollinations were performed in only one direction. All the reciprocal pollinations that were made, however, fully substantiated the results obtained in the opposite direction.

The results of these diallel pollinations expressed as percentage seed set are presented in Tables 12–14.

Table 13. *Frequency table showing fertility of the crosses depicted in Table* 12

	Percentage fertility. Class limits																
No. of crosses	0	2	4	6	10	20	30	40	50	60	70	80	90	100	100+
		2		1			6	28	36	89	115	153	172	102	76
	Sterile					Fertile									

Table 14. *Percentage fertility of the three incompatible pollinations shown in Table* 12

Genotypes	♀ plants in cross	No. of florets pollinated	Seed set	Percentage set
$1y \times 19x$	$1y$	205	0	0·0
	$19x$	149	0	0·0
$5x \times 6x$	$5x$	166	0	0·0
	$6x$	178	0	0·0
$11x \times 14y$	$11x$	199	6	3·0
	$14y$	305	22	7·2

Average fertility in incompatible crosses = 2·3 %.

Tables 12 and 13 demonstrate that 777 out of the 780 pollinations between the forty genotypes were fertile and yielded a seed set ranging from 21 to 164 %. Only three crosses proved to be incompatible, and as will be seen from Table 14 two of these ($1y \times 19x$ and $5x \times 6x$) were reciprocally cross-sterile, while the other ($11x \times 14y$) gave a seed set of

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Table 12. *Percentage fertility obtained in diallel crosses involving forty S_1S_x or S_1S_y plants derived from $S_xS_y \times S_1S_1$*

	1x	1y	2x	2y	3x	3y	4x	4y	5x	5y	6x	6y	7x	7y	8x	8y	9x	9y	10x	10y	11x	11y	12x	12y	13x	14x	14y	15x	15y	16x	16y	17x	18x	18y	19x	19y	20x	20y	21x	22x
1x	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1y	80	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2x	85	79	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2y	87	59	81	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3x	82	84	133	80	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3y	71	95	78	52	74	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4x	91	76	60	73	103	87	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4y	98	65	82	66	90	69	111	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5x	96	76	95	96	49	87	84	95	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5y	78	99	91	92	113	58	100	87	119	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6x	71	107	67	88	78	85	100	93	**0**	122	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6y	47	92	77	84	87	58	89	72	86	78	97	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7x	81	95	72	74	76	90	87	74	76	60	64	67	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7y	99	77	75	77	82	79	88	80	78	87	57	70	52	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8x	80	85	50	88	35	40	61	52	45	65	91	57	64	52	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8y	27	42	106	95	87	92	80	81	70	111	58	77	86	79	39	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9x	89	76	82	94	83	83	84	50	79	82	31	78	56	79	96	86	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9y	82	82	80	88	79	80	91	70	56	66	55	93	85	63	73	73	54	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10x	89	48	83	84	97	68	80	65	65	95	79	94	75	86	58	65	59	40	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10y	77	88	40	82	74	79	62	73	51	79	38	58	72	84	58	80	61	71	67	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
11x	89	83	63	84	91	52	83	80	118	74	60	74	103	49	87	85	80	71	64	90	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
11y	91	82	72	61	70	74	60	57	89	99	88	60	60	69	57	61	75	98	77	75	60	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12x	88	80	97	58	76	100	95	62	120	74	89	48	82	76	72	64	71	83	38	62	93	33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12y	84	92	21	89	73	106	62	38	86	90	92	50	62	66	65	79	77	93	86	49	32	73	88	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
13x	78	74	42	84	82	79	79	63	71	87	113	69	75	64	84	33	82	73	56	90	68	62	85	49	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
14x	90	31	86	32	69	78	77	66	63	89	67	84	79	79	73	86	59	86	60	58	85	86	89	66	55	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
14y	98	97	67	45	75	59	100	82	37	97	78	46	114	44	54	57	66	91	102	43	**6**	75	110	103	73	79	—	—	—	—	—	—	—	—	—	—	—	—	—	—
15x	75	103	53	116	66	100	40	51	85	80	110	56	62	76	78	56	84	70	80	62	64	79	61	59	62	86	110	—	—	—	—	—	—	—	—	—	—	—	—	—
15y	57	82	103	81	50	33	60	57	39	72	82	55	92	63	52	72	47	45	55	73	42	33	51	41	62	72	56	90	—	—	—	—	—	—	—	—	—	—	—	—
16x	79	78	76	121	87	73	48	75	82	89	70	79	91	107	66	98	84	68	87	84	75	104	130	110	79	51	98	59	67	—	—	—	—	—	—	—	—	—	—	—
16y	59	106	82	106	88	70	97	87	100	99	95	89	95	69	66	79	79	93	81	35	64	88	105	84	81	102	78	89	111	108	—	—	—	—	—	—	—	—	—	—
17x	91	116	49	84	70	45	53	63	63	158	61	64	72	77	89	72	86	75	82	42	93	70	41	100	69	46	95	66	52	92	55	—	—	—	—	—	—	—	—	—
18x	88	56	96	81	34	100	66	50	57	117	52	61	88	41	40	55	82	86	61	97	29	81	87	73	64	76	78	65	90	80	91	122	—	—	—	—	—	—	—	—
18y	79	94	149	81	51	59	78	65	66	107	104	83	50	84	91	96	32	89	67	68	64	78	102	75	74	78	90	62	32	72	54	84	83	—	—	—	—	—	—	—
19x	71	**0***	50	88	36	57	30	54	54	76	54	62	68	69	55	69	80	94	69	36	82	100	92	69	73	63	121	75	67	93	67	105	91	90	—	—	—	—	—	—
19y	83	89	51	74	71	37	87	81	100	120	87	84	82	90	66	78	89	42	112	69	106	88	66	55	74	77	89	72	57	63	77	70	95	70	66	—	—	—	—	—
20x	110	94	92	48	43	38	79	82	91	123	97	66	74	59	71	66	22	43	51	73	96	52	88	120	63	54	75	69	67	111	73	110	84	88	82	76	—	—	—	—
20y	51	88	121	76	83	124	95	98	47	92	94	82	83	97	94	89	32	86	77	90	58	80	85	84	69	53	95	56	77	71	75	65	69	92	53	111	79	—	—	—
21x	37	81	39	96	88	55	67	63	99	90	91	67	98	70	29	57	65	47	79	52	77	89	68	65	81	53	100	93	89	70	102	106	74	66	30	59	56	113	—	—
22x	89	78	111	82	83	61	92	57	56	115	164	25	92	90	102	45	93	84	93	81	62	92	76	81	108	86	82	84	91	63	50	71	100	79	74	51	92	89	82	—

* Figures in black type denote incompatible matings.

3·0 and 7·2% through pseudo-fertility when $11x$ and $14y$ were respectively used as females.

From these results it is concluded that the following S alleles were similar:

$$1y = 19x, \quad 5x = 6x, \quad 11x = 14y.$$

Of the forty unknown S allelomorphs present in this material, three were therefore represented twice, and thirty-four only once—the total number of different alleles being thirty-seven.

V. SUMMARY

1. Twenty-four out of twenty-five unrelated S_xS_y plants from a population known as English late-flowering red clover were cross-fertile as females with an S_3S_3 plant, 604(2) 3Ma (1P).

2. Forty-eight S_3S_x and S_3S_y genotypes were isolated from the twenty-four F_1 families obtained by crossing S_xS_y ♀ × S_3S_3 ♂.

3. Of the forty-eight S_x and S_y alleles, forty-one have been shown by their fertility relationships to be different.

4. Of twenty S_xS_y English broad red clover plants crossed as males with another homozygous S_1S_1 plant, 628(2) 2Ma (2P), eighteen were fully cross-fertile. The results obtained in the other two crosses were inconclusive.

5. Thirty-eight S_1S_x and S_1S_y genotypes were isolated from the F_1 families obtained from crossing twenty S_xS_y plants by S_1S_1.

6. In addition to these thirty-eight S_x and S_y alleles, two other alleles—an S_x (plant $21x$) and an S_2 (plant $22x$)—were included in the fertility tests. Thirty-seven out of a total of forty S_x and S_y alleles have been shown to be different.

REFERENCES

ATWOOD, S. S. (1944). Oppositional alleles in natural populations of *Trifolium repens*. *Genetics*, **29**, 428.

CRANE, M. B. & BROWN, A. G. (1937). Incompatibility and sterility in the sweet cherry *Prunus avium* L. *J. Pomol.* **15**, 86.

EAST, E. M. & YARNELL, S. H. (1929). Studies on self-sterility. VIII. Self-sterility allelomorphs. *Genetics*, **14**, 455.

EMERSON, S. (1939). A preliminary report of the *Oenothera organensis* population. *Genetics*, **24**, 524.

GRUBER, F. (1932). Über die Verträglichkeitsverhältnisse bei einigen selbststerilen Wildsippen von *Antirrhinum* und über eine selbstfertile Mutante. *Z. indukt. Abstamm.- u. VererbLehre*, **62**, 429.

NIJDAM, F. E. (1932). Kruisingen met *Trifolium pratense* Linn. *Genetica*, **14**, 161. (English Summary, pp. 272–4.)

WILLIAMS, R. D. (1931). Self- and cross-sterility in red clover. *Bull. Welsh Pl. Breed. Sta.* Ser. H, no. 12, p. 181.

WILLIAMS, R. D. (1939). Incompatibility alleles in *Trifolium pratense* L., their frequency and linkage relationships. *Proc. Seventh Int. Genet. Congr. Gt. Brit.* p. 316.

WILLIAMS, R. D. & SILOW, R. A. (1933). Genetics of red clover (*Trifolium pratense* L.). Compatibility. I. *J. Genet.* **27**, p. 341.

HETEROCHROMATIZATION AS A CHANGE OF CHROMOSOME CYCLE

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(With Nine Text-figures)

A survey of the problem of heterochromatization shows that we must take into account two classes of facts. On the one hand we have facts showing that a chromosome is made up of inert (heterochromatic) and active (euchromatic) regions, with different properties (Henking, 1891; Wilson, 1905–12; Heitz, 1928, 1929, 1933*a*, *b*, 1934; Muller & Painter, 1932; Darlington & La Cour, 1940, 1941; Caspersson, 1940*a*, *b*, 1941; White, 1940). On the other hand, facts have accumulated which show that the process of heterochromatization (heteropycnosis) is reversible (Mohr, 1915; McNabb, 1928; Junker, 1923; Shinke, 1937; Prokofyeva-Belgovskaya, 1937*a*, 1939*c*; Darlington & La Cour, 1940; Dobzhansky, 1944).

The work here described is intended to answer four questions:

(1) Is the conception that the chromosomes are built up of euchromatic 'euchromatin' and heterochromatic 'heterochromatin' correct?

(2) What are the causes and conditions of heterochromatization?

(3) What is the role of heterochromatization in character development?

(4) What is the nature of heterochromatization?

Structure and properties of euchromatic and heterochromatic regions

It is now definitely established that the euchromatic and heterochromatic regions do not differ significantly in their fine morphological structure. The chromosome is throughout built up of chromonemata and chromioles, these two constituents being basic both in euchromatic and heterochromatic regions.

The structure of the proximal, distal and interstitial heterochromatic regions is similar to that of the rest of the chromosome (Prokofyeva-Belgovskaya, 1937*a*, *b*, *c*, 1938, 1939*a*, *c*; Tiniakov, 1936; Frolowa, 1936*a*, *b*, *c*; Bauer, 1936). But in spite of the likeness of the basic morphological elements throughout the chromosome, the chromonemata and chromioles of the heterochromatic regions have a series of characteristic properties different from those of the euchromatic regions. These properties presumably depend on their biochemical composition, and particularly, according to Caspersson (1940*a*, *b*, 1941), on the structure of their proteins.

The characteristic, cytologically detectable properties of heterochromatic (inert) regions are as follows:

(1) Overcharging with thymonucleic acid (Caspersson, 1940; Darlington & La Cour, 1940).

(2) The capacity of all heterochromatic parts to conjugate with one another (McClintock, 1933; Prokofyeva-Belgovskaya, 1937*b*, *c*, 1938, 1939*a*; Bauer, 1936; Hinton, 1945).

(3) Mechanical weakness of their chromonemata, increasing the frequency of chromosomal rearrangement and crossing-over in them (Prokofyeva-Belgovskaya & Khvostova, 1939; Kaufmann, 1939; Prokofyeva-Belgovskaya & Belgovsky, 1943).

(4) A strong tendency to heterochromatization (heteropycnosis) (Heitz, 1928, 1929, 1933*a*, *b*).

The cytological picture of heterochromatization

Heterochromatization or heteropycnosis is a state of a chromosome section when

(1) It contains an increased amount of thymonucleic acid.

(2) Its chromonema is often twisted into a tight spiral.

(3) During most of the cell cycle it has, in distinction from other chromosomal sections, the form of a deeply staining metaphasic chromosome body. Such a form has frequently been described in different animal and plant tissues.

Heterochromatization is a condition characteristic of the mitotic stage. But whole chromosomes or definite (heterochromatic or inert) chromosome parts may assume this condition long before the nucleus as a whole enters the mitotic stage.

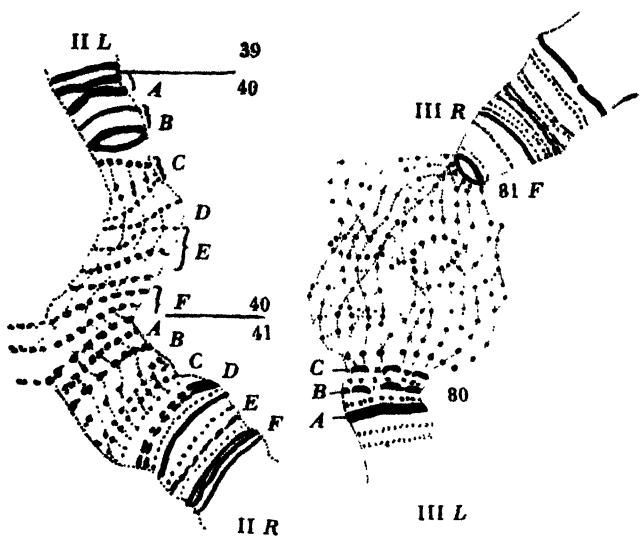

Fig. 1. Heterochromatization of the inert region and the active region situated near the inert one.

In *Drosophila*, Heitz (1933*a*, *b*, 1934) was the first to study heterochromatization. The heterochromatized sections (*X*, *Y*, and the proximal parts of II and III) have the form of typical metaphasic chromosome bodies in the prophase of larval ganglion cells. In the nuclei of salivary gland cells the heterochromatization is expressed in another way, on account of the peculiar structure of chromosomes in these cells. In comparison with the euchromatic regions where all chromomeres and chromonemata conjugate intimately with one another, the chromomeres forming bands and the chromonemata the unstaining space between them, conjugation of chromonemata is much reduced in the proximal inert regions. In inert regions, instead of bands, we see single chromomeres which sometimes contain considerable quantities of thymonucleic acid. This acid is also found in chromonemata.

One of the most typical peculiarities of the 'mitotic' condition of a chromosome is the separation or repulsion of the chromatids, connected with an increased content of thymonucleic acid. This is the basis of the cytological condition of heterochromatic regions in the salivary glands. In other words, heterochromatization in salivary glands is a shift of the cycle of certain chromosomal regions towards mitosis (Fig. 1).

Experimental part

A. *The interaction of heterochromatic and euchromatic regions: the reversibility of heterochromatization*

Is it true that heterochromatic sections always have a heterochromatic appearance, while euchromatic ones have a euchromatic appearance, or, in other words, that a chromosome is built of two substances, euchromatin and heterochromatin? To answer this question, I studied the influence of active regions on the heterochromatic structure of inert sections, and that of inert regions on the euchromatic structure of active sections.

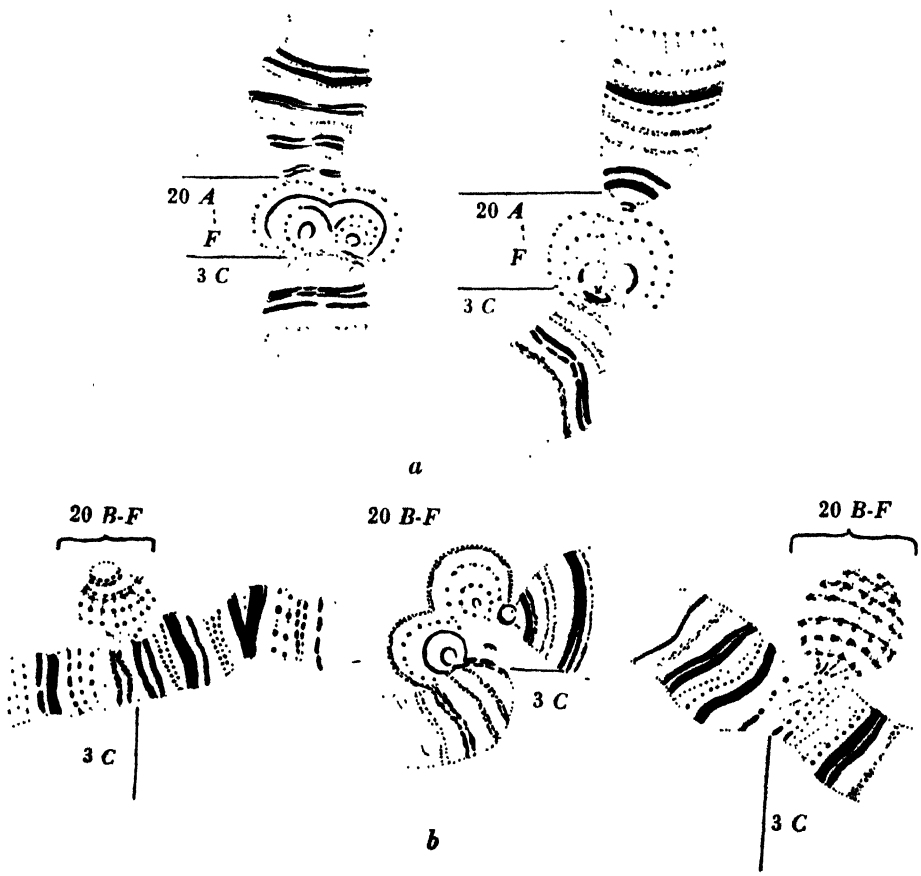

Fig. 2. The behaviour of the long inert region inserted into the active one. The origin of a deficiency. *a*, upper view; *b*, side view. The connexion of inserted inert region with a main chromosome body is very weak w^{m4}.

(1) *Change of the state of inert regions under the influence of active ones*

I investigated sc^8, w^{m4}, w^{m5}, and rst^3 stocks, in all of which a rearrangement has caused the inclusion of a part of the inert region (normally heterochromatic) in the euchromatic region. Under these conditions the heterochromatization of inert regions decreased greatly, the region inserted sometimes assuming an almost euchromatic condition, and becoming indistinguishable from the neighbouring active section. Thymonucleic acid disappears from the chromonemata, which conjugate more intimately and become invisible, while the homologous chromomeres form typical disks (Fig. 2*a*). The smaller is the inert region

inserted, the stronger is the influence of active sections upon it, as is seen on comparing the conditions of the inserted sections in the sc^8, w^{m4} and rst^3 chromosomes. This is apparently one of the causes of the euchromatic appearance of all short interstitial inert regions. When long inert regions are inserted into active ones, as in rst^3 and w^{m4}, their terminal portions often tend to conjugate, forming a small ring chromosome which is shed by the main chromosome body (Fig. 2*b*) (cf. McClintock, 1938).

(2) *Change of state of active regions under the influence of inert ones*

Inert regions have the opposite influence on active ones in their neighbourhood. I investigated a series of stocks (sc^8, w^{m5}, rst^3, w^{m4}) in which a rearrangement has brought an active region near to the inert one. In all cases the heterochromatization of the inert region spread to the neighbouring active sections, which took on a typical heterochromatic condition. Their chromonemata conjugated less intimately, and the bands assumed the form of separate chromomeres. The smaller is the active section brought close to the inert region, and the longer the latter, the stronger is the heterochromatization of the active region. The active section is sometimes so highly heterochromatized that it becomes quite indistinguishable from the neighbouring inert region (Prokofyeva-Belgovskaya, 1937*a*, *b*, 1939*c*).

These observations definitely show that the processes leading to heterochromatization or euchromatization of chromosome sections are reversible. The heterochromatic condition is not a property of heterochromatic or inert regions only, nor is the euchromatic condition confined to euchromatic or active regions. These two conditions are cyclical physiological states of a chromosomal section, and under appropriate conditions any chromosomal section may show either of the two structures.

B. *Conditions of heterochromatization*

In order to find out under what conditions heterochromatization occurs, I investigated the influences of:

(1) The neighbourhood of the centromere.
(2) The presence of an additional *Y*-chromosome.
(3) Sex.
(4) The direction of the cross.
(5) The temperature.
(6) The age of the parents.

As the states of the inert and the neighbouring active regions change similarly, we shall consider the phenomenon of heterochromatization irrespective of whether it occurs in the inert region, in the active one, or in both at once. The interdependence of the states of active and adjacent inert regions is so intimate that they must often be considered as a single region for this purpose.

(1) *The percentage of heterochromatization*

One phenomenon was always seen when the degree of heterochromatization of a particular chromosomal section was studied in different stocks and under different conditions. A section which is in the euchromatic state has the same structure in all the salivary gland nuclei of a given larva, whilst if it is in the heterochromatic state it varies considerably in different salivary nuclei of one larva.

When a section is in the heterochromatic condition in some nuclei one can hardly find two nuclei in which it has the same degree of heterochromatization. Even two neighbouring nuclei may differ very sharply, the same section being euchromatic in one and completely heterochromatic in the other (Fig. 3).

It was therefore necessary to find an index which would adequately represent the state of the nuclei in a given stock. For this purpose we chose the 'percentage of heterochromatization', which was determined as follows. I selected a sufficient number of good slides, each of which bore a pair of salivary glands from a larva of a given stock. On each slide ten nuclei in which the section investigated was in a position convenient for cytological analysis were selected under a low magnification (Apo. 10; K. 10×). This magnification

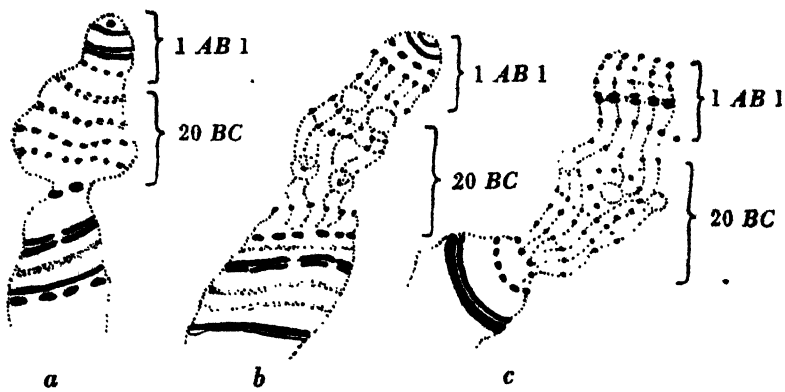

Fig. 3. The possible states of the 1*AB*1–20*ABC* region of the sc^8 chromosomes. *a*, euchromatic state; *b*, *c*, heterochromatic state.

Table 1. *State of the* 1*AB*1 (*y–ac*) 20*ABC region in the* sc^8 *chromosome,* ♀ *and* ♂ *larvae*

Genotype	No. of nuclei investigated	No. and percentage of nuclei with euchromatic region	No. and percentage of nuclei with heterochromatic region	No. and percentage of nuclei with hetero-euchromatic region
♀ $\frac{sc^8}{sc^8}$	200	69, 34·5	77, 38·5	54, 27
♂ $\frac{sc^8}{Y}$	200	168, 84	32, 16	0

allowed the marking of nuclei suitable for investigation, but was quite insufficient to determine the state of the chromosome section; so the choice of ten nuclei per slide was entirely random in this respect. The state of the section under investigation was determined under a high magnification (Apo. 90, 1·4; K. 15×). The percentage of heterochromatization is the percentage of nuclei in which the given section was in the heterochromatic condition. Numerous observations have shown that this percentage has a constant value characteristic of a given stock under definite conditions.

(2) *Influence of sex on the state of the chromosomal section*

This investigation was carried out on the *X*-chromosome of the sc^8 stock, as were most of the others. I studied the inert region 20*ABC* and the active section 1*AB*1 immediately adjacent to it, which includes the genes *y* and *ac*. The distal end of this chromosome is obviously different in the two sexes (Table 1). In males both the regions are in a well-

expressed euchromatic condition. It is often impossible to see where the active region ends and the inert one begins. This section is very rarely heterochromatized in males, and if so the degree of its heterochromatization is very low (Fig. 4).

In females the 20*ABC* and 1*AB*1 sections are highly heterochromatic in many nuclei, the degree of heterochromatization being higher in the inert region and gradually decreasing towards the distal end. The degree varies from nucleus to nucleus, and one can observe both the euchromatic and heterochromatic condition of the whole section in question, and all intermediate conditions.

The percentage of heterochromatization of the *y–ac* section is 16 % in sc^8 males, and 38·5 % in sc^8 females. In several cases (27 %) I observed differences in the structure of homologous chromosomes in the most sensitive inert region (20*ABC*) and in the adjoining active region (1*AB*1): in the chromosome derived from the father these regions had been heterochromatized, while in that derived from the mother they were in an euchromatic condition (Fig. 9). The nature of this phenomenon will be described later.

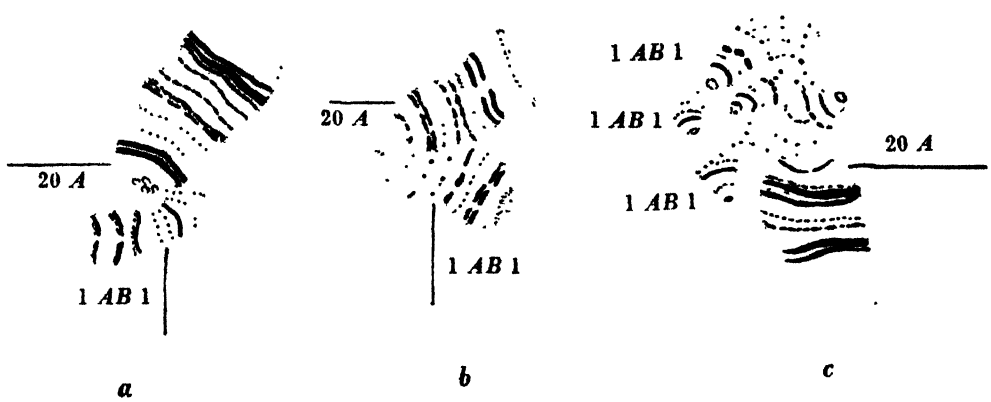

Fig. 4. Heterochromatization of the 1*AB*1–20*ABC* region of the sc^8 chromosome in males (*a*, *b*) and females (*c*).

(3) *Influence of an additional Y-chromosome on the state of the chromosome section*

I studied the structure of the 1*AB*1–20*ABC* section containing the *y* and *ac* loci of sc^8 females with an additional short arm of the *Y*-chromosome. The highly heterochromatic condition of the 20*ABC* and 1*AB*1 sections, occurring in many nuclei of ordinary sc^8 females, was strongly suppressed in this stock (Fig. 5). In the presence of an additional *Y*-chromosome these sections become more euchromatic, and the whole picture of their structure is like that observed in sc^8 males (Table 2).

(4) *Influence of the direction of the cross on the state of the chromosome section*

I studied the state of the 1*AB*1–20*ABC* section in the sc^8 chromosome of heterozygous $sc^8 \times y\ ac\ v$ females obtained in two reciprocal crosses: sc^8 ♀ × *y ac v* ♂, and *y ac v* ♀ × sc^8 ♂ (Figs. 6, 7). The first group of F_1 females received the sc^8 chromosome from their mothers, the second group from their fathers. The degree of heterochromatization of the 1*AB*1 section of this chromosome differed with the direction of the cross. In the first group its percentage of heterochromatization was 20, in the second 71, or about 3·5 times as much (Table 3).

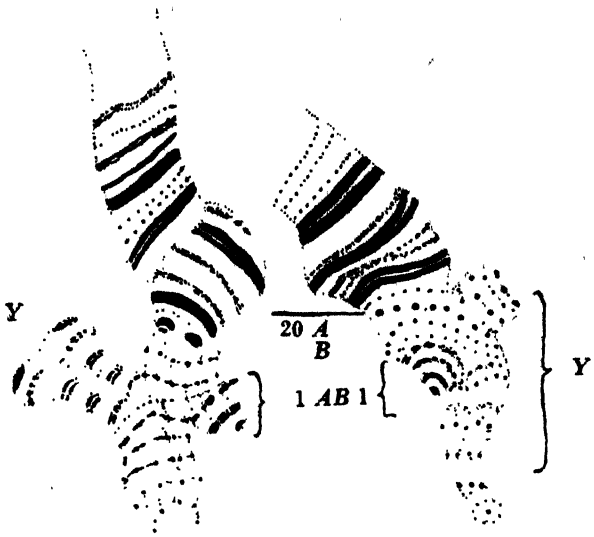

Fig. 5. The 1*AB*1–20*ABC* region of the sc^8 chromosome in females with an additional *Y*-chromosome.

Table 2. *Influence of an additional Y-chromosome on the state of the* 1*AB*1 (*y–ac*) 20*ABC region in the* sc^8 *chromosome,* ♀ *larvae*

Origin	No. of nuclei investigated	No. and percentage of nuclei with euchromatic region	No. and percentage of nuclei with heterochromatic region
sc^8 ♀ × $\widehat{XY}$* ♂	200	174, 87	26, 13
sc^8 ♀ × *y ac v* ♂	200	160, 80	40, 20

* *y ac v f* chromosome attached to the short arm of *Y*.

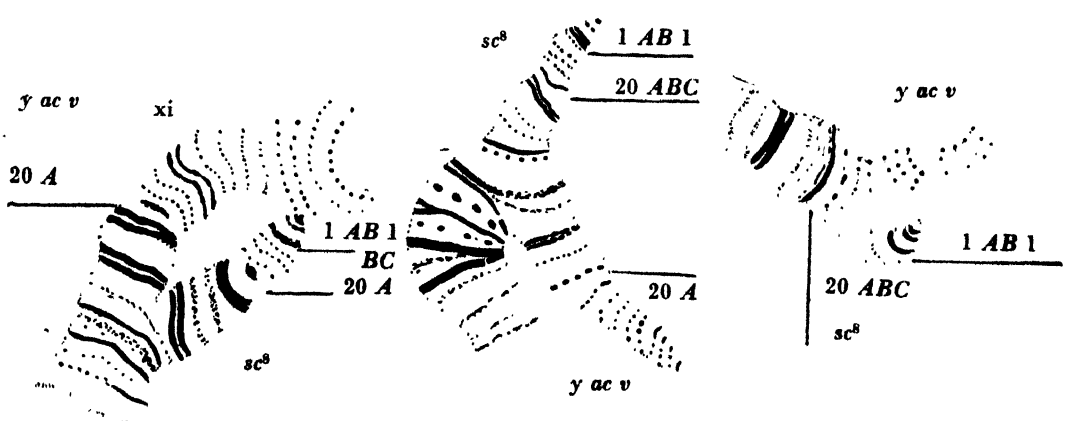

Fig. 6. The 1*AB*1–20*ABC* region of the sc^8 chromosome in ♀ sc^8 × ♂ *y ac v*, female larvae.

(5) *Influence of the centromere on the state of the chromosome section*

I investigated this influence on w^{m4}, w^{m5}, w^{mMed} and rst^3 stocks of *D. melanogaster* (Fig. 8). These stocks carry chromosomal rearrangements in which one break has occurred at the centromere of chromosome *X* or IV, the other at the *w* locus; so the active region 3*C*2 is very close to the centromere. The euchromatic state of this section is slightly disturbed in these conditions. The degree of heterochromatization occurring in the region

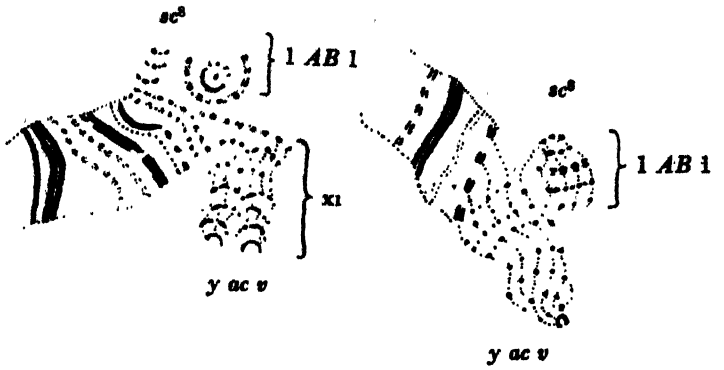

Fig. 7. The 1 *AB* 1–20 *ABC* region of the sc^s chromosome in ♀ *y ac v* × ♂ sc^s, female larvae.

Table 3. *Influence of the direction of the cross on the* 1 *AB* 1 (*y–ac*) 20 *ABC region in the* sc^s *chromosome,* ♀ *larvae*

Origin	No. of nuclei investigated	No. and percentage of nuclei with euchromatic region	No. and percentage of nuclei with heterochromatic region	Percentage of mosaics
sc^s ♀ × *y ac v* ♂	200	160, 80	40, 20	4·39
y ac v ♀ × sc^s ♂	200	58, 29	142, 71	21·92

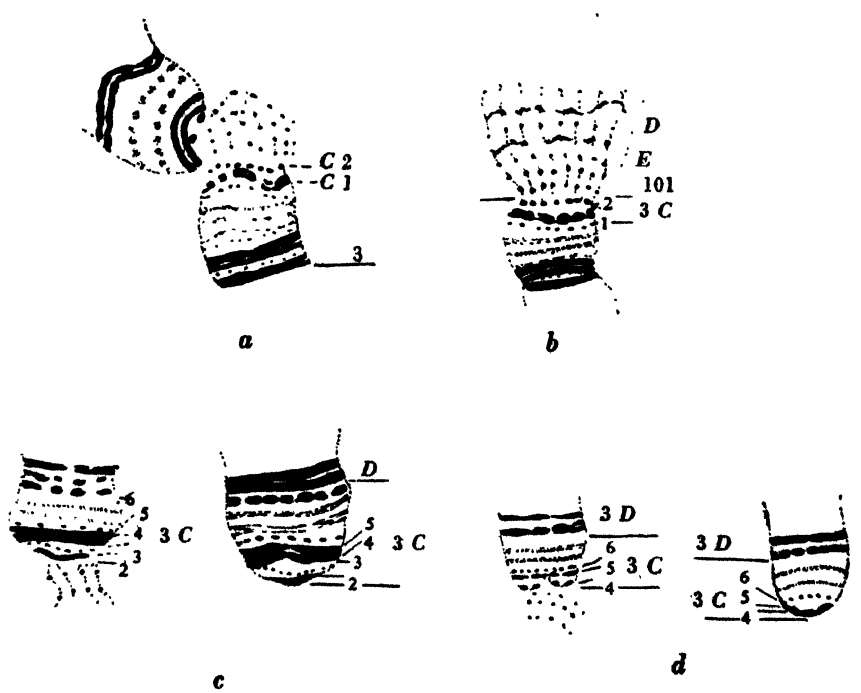

Fig. 8. *a, b.* The 3 *C* section situated near the inert region. (*a*) w^{m4}; (*b*) w^{m5}; (*c, d*) the 3 *C* section situated in the immediate neighbourhood of the centromere; (*c*) w^{m4}, (*d*) rst^s.

under the direct influence of the centromere is very low, but, nevertheless, quite definite. These observations (Table 4) suggest that the centromere is one of the intracellular factors which may cause heterochromatization of a chromosome section.

(6) *Influence of temperature on the state of the chromosome section*

The laws according to which temperature influences chromosomal regions are still far from clear. The action of temperature is probably different at different stages of development. I investigated heterozygous female larvae from the cross *y sc v* ♀ × sc^8 ♂. The eggs were laid at 25° C., and 6 hr. later portions of the cultures were transferred to 14 and 30° C.

Table 4. *Heterochromatization and mosaicism in the* w^{m4} *and* w^{m5} *lines. Influence of the centromere on the state of the* 3*C*2 (*white*) *region,* ♀ *larvae*

Stock	No. of nuclei investigated	No. and percentage of nuclei with euchromatic region	No. and percentage of nuclei with heterochromatic region	Expression of character in adult fly
w^{m4}	150	110, 73·33	40, 26·67	Dark mottled
w^{m5}	160	30, 18·75	130, 81·25	Light mottled

Table 5. *Influence of temperature on the state of the* 1*AB*1 (*y–ac*) 20*ABC region in the* sc^8 *chromosome.* ♀ *larvae* $\frac{y\ ac\ v}{sc^8}$

Temp. ° C.	No. of nuclei investigated	No. and percentage of nuclei with euchromatic region	No. and percentage of nuclei with heterochromatic region
14°	160	107, 66·9	53, 33·1
25°	200	58, 29	142, 71
30°	130	82, 63·1	48, 36·9

Table 6. *Influence of parental age on the state of the* 1*AB*1 (*y–ac*) 20*ABC region in the* sc^8 *chromosome of* ♀ *larvae from y ac v* ♀ × sc^8 ♂

Age of parents in days	Exp. 1 Euchromatic %	Exp. 1 Heterochromatic %	Exp. 2 Euchromatic %	Exp. 2 Heterochromatic %	Exp. 3 Euchromatic %	Exp. 3 Heterochromatic %
1– 5	33	67	42	58	31	69
5–10	22	78	36·5	63·5	20	80
10–15	21	79	25	75	20	80
15–20	17	83	21	79	20	80
20–25	18	82	21	79	—	—
25–30	18	82	—	—	—	—

The results are shown in Table 5. Under standard conditions (25° C.) the percentage of heterochromatization of the 1*AB*1–20*ABC* region of the sc^8 chromosome of this stock is 71. In larvae transferred to 14° C. after 6 hr. it is 33. Thus heterochromatization is suppressed when development proceeds at the low temperature. The high-temperature experiment gave very similar results. The percentage of heterochromatization in larvae transferred to 30° C. after 6 hr. was 37, and was thus again decreased.

(7) *The influence of parental age on the state of the chromosome section*

I investigated the F_1 larvae from *y ac v* × sc^8 ♂; the parents were transferred to fresh food every fifth day. Three experiments lasted 30, 23 and 20 days respectively. The *y–ac* region of the sc^8 chromosome in larval salivary glands was examined. The results are presented in Table 6.

The condition varies with the age of the parents. Their ageing results in a progressive heterochromatization of the region 20*ABC*–1*AB*1 in their progeny. There is reason to believe that ageing causes a progressive heterochromatization of the nuclei of the parents, and that this process affects the condition of the most sensitive chromosome regions in the progeny. The dependence of these regions' state in the progeny upon their condition in parents is also evidenced by the influence of the direction of the cross, and has also been shown by Belgovsky and the author (unpublished) in their study of the relation between the frequency of minute rearrangements and the degree of heterochromatization of a chromosome region.

The number of nuclei studied was 100 in each case, except in Exp. I (25–30 days), Exp. I (5–10 days), Exp. II (25–30 days) and Exp. III (15–20 days), in which 50, 88, 90 and 70 nuclei respectively were observed.

C. *Significance of heterochromatization for character development*

A transfer of active sections to the neighbourhood of inert regions regularly leads to the heterochromatization of these regions. No other cytological changes were ever observed in these cases.

After the publication of Schultz's data (Morgan, Bridges & Schultz, 1936–8) I carried out a new and extensive investigation of stocks mosaic for white (w^{m4}, w^{m5}, w^{mMed}), i.e. of stocks similar to those used by Schultz (Prokofyeva-Belgovskaya, 1939*b*, *c*). This investigation fully substantiated my first observations. No losses of active chromosome sections adjacent to inert regions were observed. The chief change is the regular assumption of the heterochromatic condition by such sections. The 3*C*2 section, containing the *w* locus in its right part, could be identified in the heterochromatic condition in every nucleus of all stocks investigated, although according to Schultz it was missing in his mosaic strains. Schultz was right only in the sense that in his cases the 3*C*2 section frequently could not be observed as an euchromatic structure, but it remained in the nucleus in the heterochromatic state. The nuclei in the stocks investigated proved very variable, some having the 3*C*2 region in the euchromatic condition, some in the heterochromatic, and others in different intermediate states.

Heitz (1928, 1929, 1932, 1933*a*, *b*, 1934) showed that heterochromatization of any chromosome section shortens its metabolic stage, since it passes through the whole cell cycle as a compact metaphasic body. This suggests that the greater the number of nuclei in which a chromosome section influencing the development of a character is heterochromatic, the more will development occur as if this section had been lost, i.e. the more strongly will a recessive character be expressed in a mosaic strain. To test this hypothesis I compared my cytological data on heterochromatization of certain chromosome sections with data on mosaicism in the same stocks obtained by other workers independently.

The degree of mosaicism for eye colour differs in the w^{m4} and w^{m5} stocks. In w^{m4} colourless ommatidia are scattered on a coloured background, so most of the eye surface shows the dominant character. In w^{m5} the recessive character prevails, the background is colourless, the pigmented ommatidia form coloured spots. In the salivary glands of w^{m4} larvae the 3*C*2 section, which plays an active part in forming pigment in ommatidia, Malphighian tubes and testes, is almost adjacent to the centromere, and is in a slightly heterochromatic state in 26·7 % of the nuclei. In the w^{m5} stock this section is near to the main bulk of the proximal inert region of chromosome IV, and in 81·25 % of nuclei is in

a well-expressed heterochromatic state (Table 4). In other words, increasing heterochromatization of this active section shifts the character towards the recessive manifestation.

This hypothesis was further tested by comparing my cytological data on heterochromatization of the 1*AB*1 section, containing the *y* and *ac* loci in the sc^8 chromosome with the genetic data of Noujdin (1944) on mosaicism in the same strain (Table 3). These data show that mosaicism is intimately connected with a change of the physiological state of the active chromosome section, namely, its conversion from the euchromatic to the heterochromatic state. This conversion depends on the approximation of this section to the inert region, which causes it to react to different genetical, developmental, and environmental conditions by changing its cycle.*

D. *Heterochromatization and crossing-over*

We saw that heterochromatization is chiefly expressed by an increased thymonucleic acid content of the chromosomes and a weakening of the conjugational properties of chromonemata. These do not conjugate with one another when in the heterochromatic state. These observations led to the suggestion that heterochromatization and euchromatization on the one hand, and the conjugation of chromosomes in meiosis on the other, depend on common causes, heterochromatization and the suppression of meiotic conjugation being phenomena of the same kind.

To check this hypothesis I compared the influence of the following factors on these two processes: temperature, inert regions, rearrangements which insert inert sections into an active region, the position of the centromere, and the parental age. I had for comparison my own cytological data on the one hand and extensive genetical data on crossing-over on the other.

(1) *Influence of temperature*

The development of young sc^8 larvae at 14 and 30° C. converts the heterochromatic state of section 1*AB*1–20*ABC* into the euchromatic one (see § B). The thymonucleic acid content of the chromonemata decreases, while their conjugational properties increase. These observations agree completely with Plough's (1917, 1921) and Mather's (1939) data on the effect of temperature on crossing-over. A decrease of temperature from 25 to 13° C. and an increase to 30° C. increases the percentage of crossing-over, which is maximal at 13 and 30° C. Plough showed that the sensitive stage at which temperature changes are effective in altering crossing-over values is that of the early oocytes, i.e. the stage at which conjugation occurs. White's (1934) studies on the influence of temperature on chiasma frequency led to similar results. Mather's more recent work fully substantiated Plough's, and also revealed some important new facts which we shall consider later.

(2) *The influence of inert regions*

The inert regions, mostly confined to the proximal ends of chromosomes, near the centromeres, persist in the heterochromatic state during most of the cell cycle. The active regions

* The numerous other cases of alterations in the normal activity of euchromatic regions approximated to the heterochromatic ones (Dubinin, 1936; Belgovsky, 1938, 1944; Demerec, 1940, 1941; Demerec & Slizynska, 1937; Kaufmann, 1942; Schultz & Caspersson, 1939; Sidorov, 1936 and others) are presumably to be explained also in terms of a change of their cycles. The inactivation of the dominant *K* gene occurring in the micronucleus of *Paramecium aurelia* (Sonneborn, 1946) must also be dependent upon the shift of the micronucleus' chromosomes towards the mitotic condition.

located near the inert ones are also in the heterochromatic condition (see §§ A, B). Thus inert regions which are heterochromatic induce heterochromatism in neighbouring active sections.

The influence of inert regions on crossing-over has been studied thoroughly. Crossing-over is strongly suppressed in the proximal ends of all chromosomes (Kikkawa, 1932; Offermann & Muller, 1932; Beadle, 1932), which is presumably due to a strong decrease in the conjugational properties of these regions.

(3) *The influence of chromosomal rearrangements involving the insertion of an inert section into the active region*

As shown in § A, when an inert region is inserted into an active one (w^{m4}, rst^3) its heterochromatization decreases considerably. Complete euchromatization was seen comparatively rarely, but heterochromatization of region 20 of the *X*-chromosome in w^{m4} and rst^3 was suppressed in almost all nuclei. The degree of heterochromatization of the inert section inserted depends largely on its size. Mather (1939) showed that the percentage of crossing-over in inserted inert sections is much increased. His data on crossing-over in the inert region 20 in rst^3 and sc^8 fully agree with our data on the heterochromatization of the same section (see also Offermann, Stone & Muller, 1931; Offermann & Muller, 1932; Beadle, 1932).

(4) *The influence of the centromere*

I showed (§ B (5)) that the centromere is an agent of heterochromatization, though a less effective one than the inert regions. When the active region 3*C*2 in the w^{m4} line is transferred nearer to the centromere, it undergoes heterochromatization, though not so marked as when it adjoins the inert region of chromosome IV in the w^{m5} line. The degree of heterochromatization varies much less between nuclei in line w^{m4} than in w^{m5}, where the sensitive inert region, responding to slight physiological differences between cells, changes the condition of the adjacent 3*C*2 region containing the *w* locus, thus considerably increasing its variability.

Mather's results (see also Graubard, 1932) on the influence of the inert regions and of the centromere on crossing-over fully agree with these observations. He concluded that, though crossing-over reduction is largely brought about by the centromere, it is not the latter which is responsible for the variation of the crossing-over percentage with temperature, but the influence of the inert regions sensitive to the changing conditions. In the sc^8 and the rst^3 lines the percentage of crossing-over is more affected by temperature in the chromosomal regions into which the inert region has been inserted by inversion than at the centromere. These regularities appear to be the same as those observed by me of the effect of the centromere and inert regions on heterochromatization, i.e. the centromere causes heterochromatization (though a slight one), while variation of heterochromatization between nuclei is related to inert regions which show a specific response to the physiological peculiarities of the cells containing them.

(5) *The influence of parental age*

It was found (§ B) that the ageing of the parents causes a progressive heterochromatization in their progeny of the most sensitive inert regions and of the active ones in their vicinity. There is reason to suppose that ageing causes a progressive heterochromatization

of the cell nuclei in the parents, and that this process tells upon the condition of the most sensitive regions in their progeny. A comparison of this evidence with that on the influence of age on crossing-over (Bridges, 1927, 1929) leads to the belief that the two phenomena are parallel. Ageing is accompanied by a reduction of the capacity of the chromosomes for conjugation and by a drop in the percentage of crossing-over.

Discussion

(1) *Heterochromatization as a change of chromosome cycle*

Considering my data on heterochromatization in connexion with our general knowledge of chromosome behaviour I conclude that heterochromatization is intimately connected with the regular chromosome changes during the cell cycle: The differences in the absorption curves of the discoidal and heterochromatic (chromocentral and interstitial) chromosome regions described by Caspersson (1940*a*, *b*, 1941) seem to be due to differences in the cycles of these regions. Heterochromatization of the inert regions and the active ones which are located in their vicinity is identical with those changes in the chromosome properties that ordinarily attend the transition of the nucleus from the metabolic stage to division. The heterochromatic chromosome sections (active or inert) are characterized by a considerable acceleration of prophasic processes and a considerable retardation of telophasic ones, leading to a great shortening of the metabolic stage of these chromosome regions. Heterochromatization is a change of chromosome cycle.

However, the conditions which may lead to this result vary with the chromosome region. If a minute disturbance in the biochemical conditions of the cell is enough to shift the cycle of the inert region in either direction, it seems to be more difficult to shift the cycle of the active chromosomal regions in the metabolic nucleus. In the cases which I studied it was only the inert regions, and those among the active ones which were brought near to them by rearrangement, which reacted visibly to changes in intracellular conditions brought about by temperature, by an extra *Y*-chromosome, by chromosomal rearrangement, by the direction of the cross, or by the age of the parents.

For the organism as a whole shifts of the chromosome cycle during the metabolic stage are of particular importance. Those chromosome regions which remain in the condition of heterochromatic mitotic bodies in metabolic nuclei will be 'inert'. Their activity and the role they play in developmental processes will thus be reduced; and if they are strongly heterochromatized they will probably be entirely eliminated from these processes as specific chromosome regions. When tissues and organs are being developed from cells containing them, an effect of physiological 'loss' of these regions will be observed, and characters determined by them will tend to show a recessive expression. The hypothesis suggested by Schultz, who explains mosaicism as a result of chromosomal deficiency for an active region, has some physiological grounds.

(2) *Genetical and environmental conditions of the chromosomal cycle*

A study of the heterochromatization of the *y–ac* section of the sc^8 chromosome in salivary gland nuclei of individuals of constitutions

$$\frac{sc^8}{\widehat{XY^*}},\quad \frac{sc^8}{Y},\quad \frac{sc^8}{y\ ac\ v},\quad \frac{sc^8}{sc^8}\quad \text{and}\quad \frac{y\ ac\ v}{sc^8}$$

* *y ac v f* chromosome attached to the short arm of the *Y*.

showed different percentages of heterochromatization, constant in each category (Table 7) and increasing in the order given. On analysing these results as to the cycle of the chromosome we find that the duration of the metabolic stage of this section is shortest in $\frac{y\ ac\ v}{sc^8}$ females, and longest in $\frac{sc^8}{\widehat{XY}}$ females. This series corresponds strictly with the types of mosaicism found in these categories of flies by Noujdin (1944). We are thus led to the conclusion that the *hereditarily conditioned degree of mosaicism of a given category of flies is due to the hereditary character of the cycle of their y–ac section.*

If we consider the facts in the light of the interpretation given to the cycle of chromosomal regions, we reach the following result: It is not irrelevant to the cycle of a given chromosomal region whether it was in a heterozygous or homozygous condition during the meiosis of the parents. A *y–ac* section which underwent meiosis in a homozygous state (in

Table 7. *Hereditary determination of the chromosome cycle of the* 1*AB*1 (*y–ac*) 20*ABC region in* sc^8 *chromosome*

Origin	Genotype	No. of nuclei investigated	Euchromatic %	Hetero-chromatic %	Hetero-euchromatic %
♀ $sc^8 \times \widehat{XY}$* ♂	$\frac{sc^8}{\widehat{XY}}$	200	87	13	0
♀ $sc^8 \times sc^8$ ♂	$\frac{sc^8}{Y}$	200	84	16	0
♀ $sc^8 \times y\ ac\ v$ ♂	$\frac{sc^8}{y\ ac\ v}$	200	80	20	0
sc^8 ♀ × sc^8 ♂	$\frac{sc^8}{sc^8}$	200	34	39	27
$y\ ac\ v$ ♀ × sc^8 ♂	$\frac{y\ ac\ v}{sc^8}$	200	29	71	0

* *y ac v f* chromosome attached to the short arm of the *Y*.

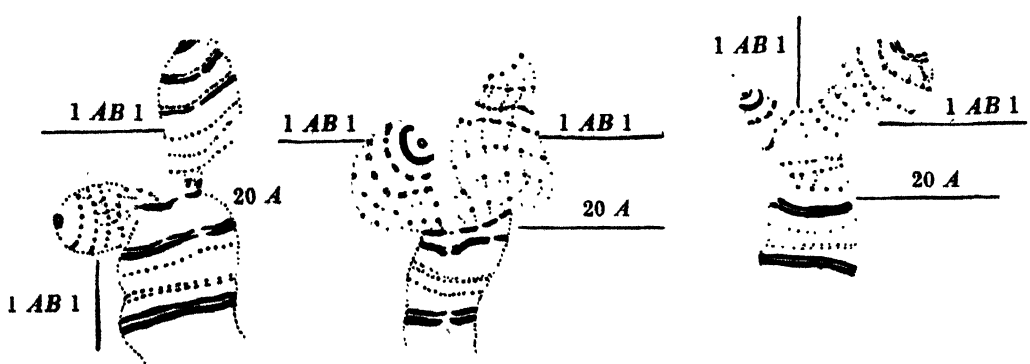

Fig. 9. The 1*AB*1–20*ABC* region of the sc^8 chromosome in female larvae.

a sc^8 chromosome derived from the mother in the cross sc^8 ♀ × *y ac v* ♂) has a longer metabolic stage in the progeny. A *y–ac* which underwent meiosis in a heterozygous state, being in a heteropyknotic condition (in a sc^8 chromosome derived from the father in the cross *y ac v* ♀ × sc^8 ♂) has a shorter metabolic stage in the progeny. In the chromosome derived from the father the *y–ac* region has been heterochromatized, in that derived from the mother it is in the euchromatic condition (Fig. 9). This cytological evidence goes to confirm the correctness of Noujdin's (1944) conclusion as to the genetic heterogeneity of chromosomes of homozygous females in the pure line sc^8.

We are thus led to the conclusion that in the case investigated the duration of the metabolic stage of the *y–ac* region of the sc^s chromosome is hereditarily determined by four factors:

(1) The biochemical nature of the *y–ac* region.

(2) Its position relative to the inert region.

(3) The condition in which it passed through meiosis in the parents.

(4) The presence in the nucleus of additional inert regions (e.g. in the *Y*-chromosome).

All these agencies, underlying the hereditary nature of the chromosome cycles, are probably of general significance. The chromosomal cycle is determined genetically, and also depends directly on environment (Darlington, 1937, 1942). In the cases which I investigated a fall of temperature to 14° C. and a rise to 30° C. acted appreciably on the heterochromatic condition of the 20 *ABC*–1 *AB*1 regions, with the loci *y* and *ac*, thus lengthening the metabolic stage of this region.

The regularities of heterochromatization, namely, (1) its increase in active regions adjoining the inert ones, (2) its suppression in inert regions inserted into active ones, (3) its varying length in active regions adjoining the inert ones, due to the latter's sensitivity, (4) its suppression at temperatures of 14 and 30° C., and (5) its increase in the progeny with the age of female parents, are all in full accord with the regularities of crossing-over (Plough, 1917, 1921; Bridges, 1927, 1929; Kikkawa, 1932; Mather, 1939).

We have seen why three different phenomena, heterochromatization, mosaicism and crossing-over, obey the same regularities: they are all underlain by a single universal process. This is the change of the chromosome cycle as a reaction to the change of genetical, developmental and environmental conditions.

Warburg (1938) and others proved the participation of different nucleotides in the constitution of the enzymes of cellular respiration. Ostern and his collaborators (1938) showed that the yeast nucleic acids may serve as precursors for the coenzyme muscle adenylic acid. These facts lead to the conclusion that the different specific nucleotides of the respiratory enzymes may have their source in nucleic acids.

On the other hand, other workers concluded that content and character of nucleic acids in the cytoplasm and nucleus depend on the cycle of the nucleus (Caspersson, 1941; Caspersson & Schultz, 1938, 1939; Brachet, 1937). According to Brachet there is a reciprocal relation between the ribose- and desoxyribose nucleic acids in the cell. As Schultz writes: 'the Janus molecule that is the gene, depending upon the material available, turns its synthesis on one face to the increase of the nucleic acid component, as at the prophases of mitosis; or conversely during the interphases, the protein component is synthesized' (Schultz, 1941).

These data, along with the results of our investigation, lead to the conclusion that the hereditary character of the metabolic process is determined by the hereditary character of the chromosome cycle.

Conclusions

1. Heterochromatization is a normal change in the chromosome cycle, which indicates a transition of chromosome regions to the 'mitotic' condition.

2. Individual chromosome regions pass through their cycle in a relatively independent way, displaying heterochromatization at a time when the main nuclear complex is in the resting condition.

3. Under definite conditions any chromosome region may become a heterochromatic one in a metabolic nucleus. By the time of metaphase the whole chromosome complex of the nucleus is reduced to this state because of the intracellular conditions.

4. The conditions of precocious heterochromatization are not the same for all chromosome regions. For sensitive inert regions and for active regions located in their close proximity, the slightest changes in the biochemical condition of the cell, of which we have no intimate knowledge, are sufficient to change their cycle to either side. The cycle of active chromosome regions remote from the centromere in the metabolic nucleus is changed with much greater difficulty.

5. The nucleus responds in the same way to changes in environmental, developmental and genetical conditions, namely, by changing its cycle. In the cases investigated, the inert regions, including 20*ABC*, and the adjoining active regions, section 1*AB*1 with the loci *y* and *ac*, responded to the alteration of developmental conditions due to temperature, the introduction of an additional *Y*-chromosome, the direction of the cross, or the age of parents, by changing their cycle.

6. Changes in the cycle of the chromosome at the stage of metabolic nucleus are of particular importance for the organism as a whole. In mosaic lines, the reduction of the metabolic stage in a given chromosome region (1*AB*1, 3*C*2) taking place as a result of heterochromatization, effects the trend of the development of the character connected with a given region towards the recessive manifestation of the character.

7. Within a single tissue, the degree of heterochromatization of the same chromosome section (1*AB*1–20*ABC*, 3*C*2–101) varies according to the cell, displaying a mosaic picture. The percentage of heterochromatization in each separate mosaic line has a rather constant value.

8. The mosaic manifestation of a character is a result of the variability of the cycles of inert regions and of regions situated near them in chromosomes of the various cells of the same tissue.

9. The hereditary nature of the duration of the metabolic stage of a given chromosome section is determined by four agents: (1) its position in the chromosome with respect to the sensitive inert region, (2) its state during the meiosis in the parents, (3) the presence in the nucleus of additional inert regions (e.g. *Y*-chromosome), and (4) its biochemical nature.

10. The duration of the metabolic stage of the *y–ac* section in the sc^8 chromosome in all the cases examined can, according to the percentage of heterochromatization, be expressed by the following series:

$$\frac{sc^8}{\widehat{XY}} > \frac{sc^8}{Y} > \frac{sc^8}{y\ ac\ v} > \frac{sc^8}{sc^8} > \frac{y\ ac\ v}{sc^8}.$$

This series fully corresponds to the types of mosaicism (as determined for the respective categories of flies by Noujdin, 1944).

11. The nuclear cycle is a subtle hereditary intracellular mechanism, the duration of whose separate stages controls the course of the morphogenetic developmental processes. It is also the mechanism by means of which the cell nucleus responds to the varying environmental condition. The actual direction of development is determined by the real course of the hereditary nuclear and cellular cycles under definite developmental conditions.

12. Three phenomena, heterochromatization, crossing-over, and mosaicism, are thus

found to obey the same regularities, being underlain by a common universal process—the variation of the chromosome cycle, as its response to varying developmental conditions.

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REFERENCES

BAUER, H. (1936). Structure and arrangement of salivary gland chromosomes in *Drosophila* species. *Proc. Nat. Acad. Sci., Wash.*, **22**, 216–22.

BEADLE, G. W. (1932). A possible influence of the spindle fibre on crossing-over in *Drosophila*. *Proc. Nat. Acad. Sci., Wash.*, **18**, 160–5.

BELGOVSKY, M. L. (1938). Influence of inert regions of chromosomes on the frequency of occurrence and type of changes in the adjacent active sections. *Bull. Acad. Sci. U.R.S.S.*, Sér. biol., pp. 1017–36.

BELGOVSKY, M. L. (1944). On the causes of mosaicism associated with heterochromatic chromosome regions. *J. Gen. Biol.* **6**.

BRACHET, J. (1937). Remarques sur la formation de l'acide thymonucleique pendant le développement des oeufs à synthèse partielle. *Arch. de Biol.* **48**, 529.

BRIDGES, C. B. (1927). The relation of the age of the female to crossing-over in the third chromosome of *Drosophila melanogaster*. *J. Gen. Physiol.* **8**, 689–700.

BRIDGES, C. B. (1929). Variation in crossing-over in relation to age of female in *Drosophila melanogaster*. *Publ. Carneg. Instn*, no. 399, pp. 63–89.

CASPERSSON, T. (1940*a*). Die Eiweissverteilung in den Strukturen des Zellkerns. *Chromosoma*, **1**, 562–604.

CASPERSSON, T. (1940*b*). Über Eiweisstoffe im Chromosomgerüst. *Naturwissenschaften*, **28**, 514.

CASPERSSON, T. (1941). Studien über den Eiweissumsatz der Zelle. *Naturwissenschaften*, **29**, 33.

CASPERSSON, T. & SCHULTZ, J. (1938). Nucleic acid metabolism of the chromosomes in relation to gene reproduction. *Nature, Lond.*, **142**, 294.

CASPERSSON, T. & SCHULTZ, J. (1939). Pentose nucleotids in the cytoplasm of growing tissues. *Nature, Lond.*, **143**, 602.

DARLINGTON, C. D. (1937). *Recent Advances in Cytology*. London.

DARLINGTON, C. D. (1942). Chromosome chemistry and gene action. *Nature, Lond.*, **149**, 66.

DARLINGTON, C. D. & LA COUR, L. (1940). Nucleic acid starvation of chromosomes in *Trillium*. *J. Genet.* **40**, 185–213.

DARLINGTON, C. D. & LA COUR, L. (1941). The detection of inert genes. *J. Hered.* **32**, 115–22.

DEMEREC, M. (1940). Genetic behavior of euchromatic segments inserted into heterochromatin. *Genetics*, **25**, 618–27.

DEMEREC, M. (1941). The nature of changes in the white-notch region of the *X*-chromosome of *Drosophila melanogaster*. *Proc. Seventh Int. Genet. Congr.* pp. 99–103.

DEMEREC, M. & SLIZYNSKA, H. (1937). Mottled white 258–18 of *Drosophila melanogaster*. *Genetics*, **22**, 641–9.

DOBZHANSKY, TH. (1944). Distribution of heterochromatin in the chromosomes of *Drosophila pallidipennis*. *Amer. Natur.* **78**, 193.

DUBININ, N. P. (1936). A new type of position effect. *Biolog. Zhurn.* **5**, 851–4.

FROLOWA, S. L. (1936*a*). Structure of the nuclei in the salivary gland cells of *Drosophila*. *Nature, Lond.*, **137**, 319.

FROLOWA, S. L. (1936*b*). Struktur der Kerne in den Speicheldrüsen von *Drosophila sulcata* Stert. *Bull. Biol. Méd. exp.* **2**, 98–101.

FROLOWA, S. L. (1936*c*). Struktur der Kerne in den Speicheldrüsen einiger *Drosophila*-Arten. *Biol. Zh., Mosk.*, **5**, 271–92.

GRAUBARD, M. A. (1932). Inversion in *Drosophila melanogaster*. *Genetics*, **17**, 81–105.

HEITZ, E. (1928). Das Heterochromatin der Moose. I. *Jb. wiss. Bot.* **69**, 762–818.

HEITZ, E. (1929). Heterochromatin, Chromozentren, Chromomeren. *Ber. dtsch. bot. Ges.* **47**, 274–84.

HEITZ, E. (1932). Die Herkunft der Chromocentren. Dritter Beitrag zur Kenntnis der Beziehung zwischen Kernstruktur und qualitativer Verschiedenheit der Chromosomen in ihrer Längsrichtung. *Planta*, **18**, 571–636.

HEITZ, E. (1933*a*). Über totale und partielle somatische Heteropyknose, sowie strukturelle Geschlechtschromosomen bei *Drosophila funebris*. *Z. Zellforsch.* **19**, 720–42.

HEITZ, E. (1933*b*). Die somatische Heteropyknose bei *Drosophila melanogaster* und ihre genetische Bedeutung. *Z. Zellforsch.* **20**, 237–87.

HEITZ, E. (1934). Eine Beziehung zwischen der genetischen und strukturellen Längsdifferenzierung des *X*-Chromosomes von *Drosophila melanogaster*. *Z. ind. Abst. u. Vererb. Lehre*, **67**, 216–17.

HENKING, H. (1891). Untersuchungen über die ersten Entwicklungsvorgänge in den Eiern der Insekten. II. *Z. wiss. Zool.* **51**, 685–736.

HINTON, T. (1945). A study of chromosome ends in salivary gland nuclei of *Drosophila*. *Biol. Bull.* **88**, 144.

JUNKER, H. (1923). Zytologische Untersuchungen an den Geschlechtsorganen der Steinfliege *Perla marginata*. *Arch. f. Zellf.* **17**, 185.

KAUFMANN, B. P. (1939). Distribution of induced breaks along the *X*-chromosome of *Drosophila melanogaster*. *Proc. Nat. Acad. Sci., Wash.*, **25**, 571–7.

KAUFMANN, B. P. (1942). Reversion from roughest to wild type in *Drosophila melanogaster*. *Genetics*, **27**, 537–49.

KIKKAWA, H. (1932). Studies on the mechanism of crossing-over in *Drosophila*, with special reference to the phenomenon of interference. *Cytologia, Tokyo*, **3**, 285–332.

MATHER, K. (1939). Crossing-over and heterochromatin in the *X*-chromosome of *Drosophila melanogaster*. *Genetics*, **24**, 413.

MCCLINTOCK, B. (1933). The association of non-homologous parts of chromosomes in the mid prophase of meiosis in *Zea mays*. *Z. Zellforsch.* **19**, 191.

MCCLINTOCK, B. (1938). The production of homozygous deficient tissues with mutant characteristics by means of the aberrant mitotic behaviour of ring-shaped chromosomes. *Genetics*, **23**, 315–76.

MCNABB, J. W. (1928). A study of the chromosomes in meiosis, fertilization and cleavage in the grasshopper egg (Orthoptera). *J. Morph.* **45**, 47–93.

MOHR, OTTO (1915). Sind die Heterochromosomen wahre Chromosomen? Untersuchungen über ihr Verhalten in der Ovogenese von *Leptophyes punctatissima*. *Arch. Zellforsch.* **14**, 151–76.

MORGAN, T. H., BRIDGES, C. B. & SCHULTZ, J. (1936–8). Investigations on the constitution of the germinal material in relation to heredity. *Yearb. Carneg. Instn*, nos. 35–7.

MORGAN, T. H., SCHULTZ, J. & CURRY, V. (1939–40). Investigations on the constitution of the germinal material in relation to heredity. *Yearb. Carneg. Instn*, nos. 38–9.

MULLER, H. J. & PAINTER, T. (1932). The differentiation of the sex chromosomes into genetically active and inert regions. *Zeit. ind. Abst. u. Vererb. Lehre*, **63**, 316.

NOUJDIN, N. I. (1944). The regularities of the influence of heterochromatin on mosaicism. *J. Gen. Biol.* **6**.

OFFERMANN, C. A. & MULLER, H. J. (1932). Regional differences in crossing-over as a function of the chromosome structure. *Proc. Sixth Int. Congr. Genet.* **2**, 143–5.

OFFERMANN, C. A., STONE, W. S. & MULLER, H. J. (1931). Causes of inter-regional differences in cross-over frequency, studied in individuals homozygous for gene rearrangements. *Anat. Rec.* **51**, 109.

OSTERN, P., TERSZAKOWEĆ, J. & HUBL, ST (1938). Über die Phosphorylierung des Adenosins durch Hefe und die Bedeutung dieses Vorgangs für die alkoholische Gärung. *Hoppe-Seyl. Z.* **255**, 104–25.

PLOUGH, H. H. (1917). The effect of temperature on linkage in the second chromosome of *Drosophila*. *Proc. Nat. Acad. Sci., Wash.*, **3**, 553–5.

PLOUGH, H. H. (1921). Further studies on the effect of temperature on crossing-over. *J. Exp. Zool.* **32**, 187–202.

PROKOFYEVA-BELGOVSKAYA, A. A. (1937*a*). Observations on the structure of chromosomes in the salivary glands of *Drosophila melanogaster*. *Bull. Acad. Sci. U.R.S.S.*, Sér. biol., no. 2, pp. 393–426.

PROKOFYEVA-BELGOVSKAYA, A. A. (1937*b*). Inert regions in distal ends of the chromosome of *Drosophila melanogaster*. *Bull. Acad. Sci. U.R.S.S.*, Sér. biol., no. 3, pp. 719–24.

PROKOFYEVA-BELGOVSKAYA, A. A. (1937*c*). Structure of the *Y*-chromosome in salivary-gland's cells of *Drosophila melanogaster*. *Genetics*, **22**, 94–104.

PROKOFYEVA-BELGOVSKAYA, A. A. (1938). Inert region in the subterminal part of the *X*-chromosome of *Drosophila melanogaster*. *Bull. Acad. Sci. U.R.S.S.*, no. 1, pp. 97–103.

PROKOFYEVA-BELGOVSKAYA, A. A. (1939*a*). Inert regions in the inner part of the *X*-chromosome of *Drosophila melanogaster*. *Bull. Acad. Sci. U.R.S.S.*, no. 3, pp. 362–70.

PROKOFYEVA-BELGOVSKAYA, A. A. (1939*b*). Cytological mechanism of mosaicism and of chromosome rearrangement. *C.R. Acad. Sci. U.R.S.S.* **22**, 274–7.

PROKOFYEVA-BELGOVSKAYA, A. A. (1939*c*). Cytological study of the breaks at the white locus of the *X*-chromosome of *Drosophila melanogaster*. *Bull. Acad. Sci. U.R.S.S.*, no. 2, pp. 215–27.

PROKOFYEVA-BELGOVSKAYA, A. A. & BELGOVSKY, M. L. (1943). The change of the cross-over properties of a chromosome under the influence of mutation. *C.R. Acad. Sci. U.R.S.S.* N.S. **38**, 281–4.

PROKOFYEVA-BELGOVSKAYA, A. A. & KHVOSTOVA, V. V. (1939). Distribution of breaks in the *X*-chromosome of *Drosophila melanogaster*. *C.R. Acad. Sci. U.R.S.S.* **23**, 269–71.

SCHULTZ, J. (1936). Variegation in *Drosophila* and the inert chromosome regions. *Proc. Nat. Acad. Sci. Wash.*, **22**, 27–33.

SCHULTZ, J. (1941). The evidence of the nucleoprotein nature of the gene. *Cold Spr. Harb. Symp.* **10**, 55.

SCHULTZ, J. & CASPERSSON, T. (1939). Heterochromatic regions and the nucleic acid metabolism of the chromosome. *Arch. Zellforsch.* **22**, 650–4.

SHINKE, N. (1937). An experimental study on structure of living nuclei in the resting stage. *Cytologia, Fujii, Jubil.* vol. I.

SIDOROV, B. N. (1936). The mutability of yellow, achtate and scute in lines scute^{8} and yellow^{3p}. *Biol. Zh., Mosk.*, **5**, 3–26.

SONNEBORN, T. M. (1946). Inert nuclei inactivity of micronuclear genes in variety 4 of *Paramecium aurelia*. *Genetics*, **31**, 231.

TINIAKOV, G. G. (1936). On inert parts and the general morphology of salivary gland chromosomes. *Bull. Biol. Méd. exp. U.R.S.S.* **2**, 209–11.

WARBURG, O. (1938). Chemische Konstitution von Fermenten. *Ergebn. Enzymforsch.* **7**, 210–45.

WHITE, M. J. D. (1934). The influence of temperature on chiasma frequency. *J. Genet.* **29**, 203–15.

WHITE, M. J. D. (1940). The heteropycnosis of sex chromosomes and its interpretation in terms of spiral structure. *J. Genet.* **40**, 67–82.

WILSON, E. (1912). Studies on chromosomes. I–VIII. *J. Exp. Zool.* **13**, 345–448.

A DUPLICATION AND A DEFICIENCY IN *OENOTHERA*

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(With Six Text-figures)

1. Introduction

In the study of the P^s-S position effect in *Oenothera blandina* (Catcheside, 1946, Table 1) a number of exceptional plants with modified expressions of the position effect abnormal in their inheritance were found. The evidence shows that in these plants the P^s-S region of the chromosome was duplicated. In addition corresponding deficiencies were found.

The term 'duplication' has been used for any genetic situation in which a small part of a chromosome has undergone doubling within the haploid set. A considerable variety of

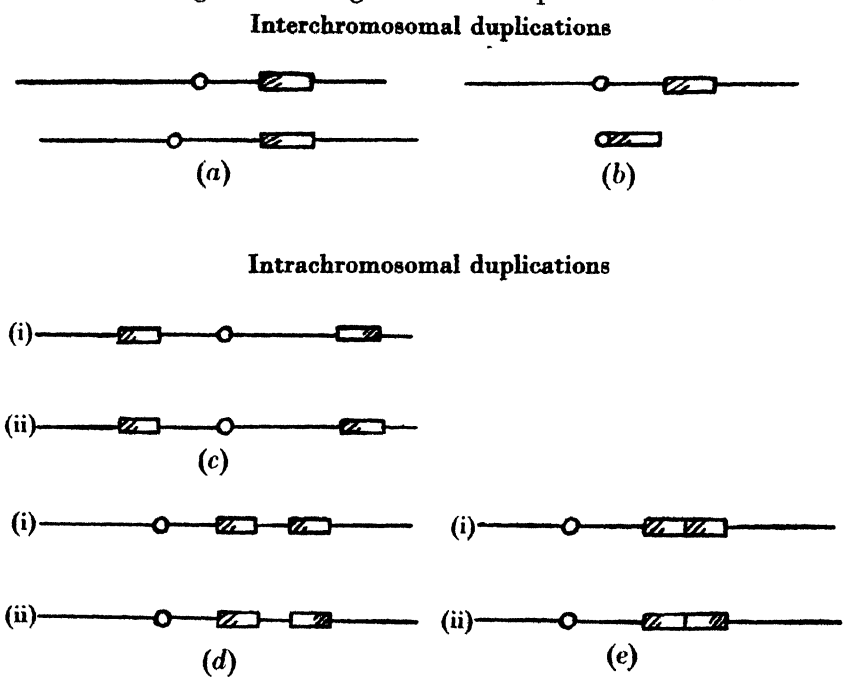

Fig. 1. Principal types of duplication possible in chromosomes. The centromere is shown by means of a circle and the duplicate segments by oblongs, the two ends of which are distinguished by shading.

such duplications may be expected. The more frequently studied cases are those in which the duplication is *inter-chromosomal*, that is, the two duplicate chromosome portions are attached to separate centromeres. Two cases may be distinguished in this group. In one (Fig. 1*a*) the duplication is inserted into another normal chromosome different from that from which it has been derived. Cytological evidence of this is provided by pairing and chiasma formation in haploids, e.g. of *Oenothera* (Catcheside, 1932). An example studied in *Drosophila melanogaster* by Dobzhansky (1934) is *Dp* (1; 3) 126, in which a section of the *X*-chromosome from *r* to the right of *B* has been inserted into the third chromosome between *st* and *cu*. In the other type (Fig. 1*b*) the duplication is present as an extra fragment chromosome, as in certain clones of *Fritillaria imperialis* (Darlington, 1930) and numerous genetic stocks of *Drosophila melanogaster* such as *Dp* (1; *f*) 100 which has a

large duplication two-thirds the length of the *X*-chromosome (Morgan, 1938). Bridges & Brehme (1944) list many others.

Less frequently studied, but no less important, are those cases in which the duplication is *intra-chromosomal*, with the two portions attached to the same centromere. The well-known 'repeats' observed in the salivary chromosomes of *D. melanogaster* (Bridges, 1935) come into this group. In these cases, the duplicated portions may be either *non-contiguous* or *contiguous* (Fig. 1*e*). Non-contiguous duplications may be within the same chromosome arm (Fig. 1*d*) or in separate chromosome arms (Fig. 1*c*). Finally, the two portions may be arranged in the same sense ((i) in Fig. 1) with respect to the centromere and be *direct*, or in opposite, i.e. relatively inverted, senses ((ii) in Fig. 1) and be *reverse*. Thus in the intra-chromosomal group six types of duplication should be distinguishable. In *D. melanogaster*, the two contiguous types are represented by Bar (Bridges, 1936), Hairy wing (Demerec & Hoover, 1939) and Confluens (Schultz, 1941), which are direct, and by eyeless-Dominant (Bridges, 1935) and Star-asteroid (Lewis, 1945), which are reverse.

Table 1. *Products of exceptional types of pairing and crossing-over in intra-chromosomal duplications*

	Products of	
Type of intra-chromosomal duplication	Intra-chromosomal pairing with crossing-over between duplicate segments in same chromatid	Unequal pairing of two homologous chromosomes *or* Intra-chromosomal pairing with crossing-over between duplicate segments in sister chromatids
Inter-arm direct	Inversion of region between duplicate segments	Duplication-deficiency chromosomes for regions distal to duplicate segments
Inter-arm reverse	Centric ring chromosomes of region between duplicate segments + acentric rod	Dicentric + acentric chromosomes
Intra-arm non-contiguous		
Direct	Acentric ring of region between duplicate segments + centric deficient chromosome	Contiguous direct duplication of region including duplicate segments + centric deficient chromosome
Reverse	Inversion of region between duplicate segments	Dicentric + acentric chromosomes
Intra-arm contiguous		
Direct ('Tandem')	Acentric ring + centric chromosomes with duplication singularized	Contiguous direct triplication + singularized chromosome
Reverse	Inversion of region	Dicentric + acentric chromosomes

Some duplications can arise by crossing-over from others. In general this is a special property of the intrachromosomal duplications, but in *D. melanogaster*, inter-arm duplications of the *X*-chromosome may arise from interchromosomal ones. Thus *Dp* (1; 1) 100, described by Morgan (1938), has the duplication attached to the right end of the *X*; it arose by crossing-over in an attached-*X* female carrying the duplication fragment of *Dp* (1; *f*) 100.

The intra-chromosomal duplications have various important properties, dependent upon pairing of the homologous duplicate segments in various ways followed by chiasma formation between the paired duplicate segments. The consequences are summarized in Table 1, the behaviour particular to our present case being described in more detail in § 4.

2. Proof of the duplication in *Oenothera blandina*

The origin of the plants, later interpreted as carrying a *P-S* duplication, was as follows. When $A\ P^r/+P^s$ plants, showing a P^r sepal colour variegation with deep red patches on a green background (Fig. 1*d* of previous paper), were pollinated by $+P^s$ plants, a small

fraction of the progeny proved to be of a novel and unexpected type, which may be called X. Their sepals had deep red patches on a medium red background (Fig. 1*e* of previous paper) and the gametes carrying the abnormality were pollen lethal and nearly inviable as megaspores. These plants had 14 chromosomes and possessed the interchange, and were not distinguishable cytologically from the normal interchange plants.

If we consider the petal colour phenotypes in type X plants coming from interchange heterozygotes with different *S* locus compositions and receiving $+P^s\,s$ in their regular 3.4 chromosome the following facts appear. The X plants from $A\,P^r\,S/+P^s\,S\times+P^s\,s$ have yellow petals (X-1); those from $A\,P^r\,S/+P^s\,s\times+P^s\,s$ have variegated yellow and sulphur petals (X-2); and those from $A\,P^r\,s/+P^s\,S\times+P^s\,s$ have yellow petals (X-3). Now the X-3 plants must have an *S* gene in the 3.11 chromosome and this chromosome must have received it from the 3.4 chromosome of the parent by crossing-over. Equally the X-2 plants have an S gene in their 3.11 chromosome, this time from the 3.11 chromosome of the parent. While X-2 and X-3 each have an *S* gene in their 3.11 chromosomes, there must be a difference between their 3.11 chromosomes since the phenotypic appearance of the petals is different in the two kinds of plants.

The explanation is finally shown when we consider progenies of X-1 plants that have been pollinated by $+P^s\,s$ (Table 4A). In this case, numerous cross-overs of *S* from the 3.11 chromosome to the 3.4 chromosome result in yellow-petalled non-interchange plants. The complementary cross-overs, in which chromosome 3.11 should have received *s* from chromosome 3.4, yield X-type plants with variegated yellow and sulphur petals. Therefore the 3.11 chromosomes of these cross-over plants still possess each an *S* gene, although they have each lost an *S* gene by crossing-over. The inference is that each of these 3.11 chromosomes possessed initially two *S* genes. *Hence, the S locus in the* 3.11 *chromosome of the* X-*type plants is duplicated.* Thus X-1 plants were $S\,S/s$, X-2 plants $S\,s/s$ and X-3 plants $s\,S/s$, the locus of the interchange break being to the left of the duplication.

Further, the *S* gene is subject to position effect variegation only when it is situated in the proximal, or left-hand, segment of the duplication. This is shown by different phenotypes of the X-2 and X-3 plants.

That the *P* locus is also duplicated cannot be proved quite so directly. The medium red background to the sepals is circumstantial evidence that the plants carry a P^s gene on each of their 3.11 and 3.4 chromosomes, while the deep red patches are evidence of the presence of P^r in addition. Further evidence is provided by the facts that X plants arising from $A\,P^s\,S/+P^r\,S\times+P^s\,s$ have wholly deep red sepals and yellow petals, while those from $A\,P^s\,S/+P^r\,s\times+P^s\,s$ have wholly deep red sepals and variegated petals. The indication is that these X plants have P^r in the distal segment of the duplication, where it is not subject to variegation. The most satisfactory evidence would be provided by the progenies of the duplication $A\,P^r\,P^r/+P^s$, but these plants have not yet been available.

Recurrence of the same structural abnormality requires a regular origin by exceptional pairing and crossing-over. The origin of a duplication is most easily accounted for by unequal crossing-over, and this in turn requires a pre-existing duplication, i.e. a structural peculiarity that permits asymmetrical pairing. In the present case the duplication must be within the same arm and be direct, otherwise the products of unequal pairing (see Table 1) would be functionally or mechanically inviable. It is most probable that the pre-existing duplication was of the non-contiguous type, otherwise the normal chromosomes would already have a *P*-*S* duplication and the X types would therefore be triplications.

Complementary to the duplication we should expect to recover a deficient chromosome, provided it is haplo-viable. In the cultures, a deficiency of the P-S region has appeared several times. It provides corroborative evidence for the suggested method of origin of the duplication.

The 3.11+4.12 interchange, from heterozygotes of which the duplication arises, originated through X-radiation of a normal pollen grain having chromosomes 3.4 and 11.12. It is necessary to decide whether the non-contiguous duplication occurs naturally in *Oenothera blandina* or whether it was instead induced at the same time as the interchange. In favour of the former is the fact that the deficiency arises rarely from normal *blandina* itself which has never been in association with the interchange. It is unknown whether the duplication also appears in normal *blandina*, mainly because there has been no ready method available for its recognition. It is to be hoped that some fairly reliable method of recognition through an effect on pollen fertility may become available.

3. Behaviour of the P^s-S deficiency

Plants heterozygous for this deficiency are phenotypically distinct from normal *O. blandina* most conspicuously by their possession of nearly green buds. The whole plant is of a markedly brighter green and has a characteristically different habit. The leaves are relatively broader, and the plant is branched at about half-way up the stem rather than from

Table 2. *Progenies of deficiency plants*

Plant	Genotype	Type of progeny	Progenies
70/40 III 2	$+Df/+P^s$	F_2	27 P^s P^s *blandina*
		Backcross by P^s P^s *blandina*	27 P^s P^s *blandina*
93/34 II 3	$+Df/+P^s$	F_2	31 P^s P^s *blandina*
69/40 V 13	$A\ Df/+P^s$	F_2	27 P^s P^s *blandina*
		Backcross by P^s P^s *blandina*	26 P^s P^s *blandina*, 1 trisomic

the base. The anthers are thin and half the pollen is sterile, being empty and smaller than the normal pollen. Normal *blandina* is homozygous for the gene P^s and therefore shows broad medium red stripes on the sepals; the flower buds are thus predominantly a medium red colour. Plants heterozygous for the deficiency have green sepals with a slight red flush in their upper halves. Such plants, hemizygous for P^s, which have nearly green flower buds at the beginning of the season, become somewhat redder later in the season when the weather is cooler. There is little or no difference in bud pigmentation between plants hemizygous for P^s and those that are P^s P or P^s p. A deficiency for the P^s locus gives an effect similar to the P and p allelomorphs in respect to bud colour.

The deficiency also includes the neighbouring S locus. S S plants and hemizygous S plants have yellow petals, while s s and hemizygous s plants have sulphur coloured (pale yellow) petals.

Plants heterozygous for the deficiency fail to transmit it either through the pollen or through the embryo sac so far as is known. However, rather small numbers of progeny have so far been grown. The available data are collected in Table 2. The 50% of bad pollen evidently represents the expected deficiency pollen which has been killed by the deficiency. Since the deficiency is completely lethal to the pollen, the selfed families may be employed as though they were backcrosses to normal *blandina* as pollen parent. Hence there were no deficient eggs in a sample of 138 tested. It is improbable ($P=0{\cdot}01$ level) that more than 5·2 such eggs could have been expected and therefore it is probable that

deficient eggs are not more than 3·8% of all functional eggs in plants heterozygous for the deficiency.

The absence of functional deficiency embryo-sacs requires a special explanation. The mere fact that zygotes heterozygous for the deficiency can arise shows that the deficiency is not lethal to the megaspore, embryo-sac or egg *per se*. Probably, instead, the deficiency megaspore is at a total (or nearly total) disadvantage relative to a normal gamete in competition for the production of an embryo-sac. A Renner effect may be expected or else a polarized segregation. Relative to a duplication gamete, however, it may be at a less serious disadvantage. In the origin of the deficiency by unequal crossing-over at megasporogenesis a corresponding duplication should arise, and the two would come into competition in embryo-sac formation in at least a proportion of the megaspore tetrads in which they originate.

Similar plants with a P^s-S deficiency in an interchange, 3.11 chromosome also occur (Table 2). Occasionally chimerical plants that are partly deficient and partly normal have also been observed. None has so far been analysed by growing progenies from it, but in all cases the appearance of the two parts of the plant suggests that the deficient sector has been derived from the normal sector by somatic loss of the P^s-S region. In no case was a whole chromosome missing, so it appears that we may be dealing with a case of intrachromosomal somatic crossing-over of a type analogous to that postulated in Table 1.

4. Behaviour of the P^s-S duplication

So far the duplication has been found only in interchange, 3.11 chromosomes where it may be detected by the reaction of the distal segment with the proximal segment in which P^s (or P^r) and S are subject to position effects. If the interchange is represented by the letter A and the normal chromosomes by +, a plant that is $A\ P^s/+P^s$ or $A\ P^r/+P^s$ shows variegation in its sepal colour with patches of red sharply separated by green tissue. In $A\ P^s/+P^s$ the red is a medium tone, while in $A\ P^r/+P^s$ the red is a deep one. Similarly in $A\ S/+s$ the petals are variegated yellow and pale yellow (sulphur colour), the boundaries again being sharply defined.

When the P^s-S segment is duplicated, sixteen gametic types of the duplication (Table 5) may be expected, depending upon the allelomorphs present at the four available loci. Several have been recognized and others may have occurred, but the evidence respecting them is inconclusive. Plants heterozygous for the interchange-with-duplication (hereafter referred to as A-Dp) are not distinguishable by any conspicuous characters of habit or foliage from corresponding plants lacking the duplication (referred to as A). There are slight differences, but it is doubtful whether they allow a reliable diagnosis. Two of the genotypes that have been identified are $A\ P^r\ S\ P^s\ S/+P^s$ and $A\ P^r\ S\ P^s\ s/+P^s\ s$. Both produce a type of sepal variegation in which the dark red areas of epidermal tissue are separated by medium red tissue instead of green tissue. It is as though the P^s genes painted in a medium red background to the dark red patches due to the P^r gene. This is the phenotypic effect that led ultimately to the recognition of the duplication.* The former genotype produces entirely yellow petals, the latter produces variegated yellow-sulphur petals.

* I am greatly indebted to Dr K. Mather (John Innes Horticultural Institution) for his suggestion of what has proved to be the composition of these plants. His idea was based upon knowledge of the patterns produced by two different flower-variegating genes together in the same plants of *Antirrhinum*.

A-Dp appears to be lethal to pollen grains. Heterozygotes, *A-Dp*/+, have considerably in excess of 50% bad pollen, actually about 60–70% bad compared with the 20% found in the normal interchange heterozygotes, *A*/+. In confirmation is the fact that *A-Dp* gametes are not transmitted through the pollen (Table 3B). Further *A-Dp* megaspores are at a marked disadvantage in competition with + megaspores, for *A-Dp* gametes constitute only a small minority of the functional eggs (Table 3A). This is one of the factors that makes work with the duplication rather troublesome.

Table 3A. *Progenies of A-Dp* P^r P^s/+P^s × +P^s/+P^s

Family	Parents	Progeny			
		+P^s/+P^s	+P^r/+P^s	*A* P^s/+P^s	*A-Dp* P^r P^s/+P^s
12/41	72/40 XI 8 × 1/40 I 1	135	1	1	17
23/42	72/40 XI 8 × 1/40 I 2	106	.	.	8
22/43	23/42 I 1 × 26/42 I 3	36	1	1	1
23/43	23/42 I 1 × 26/42 I 3	11	.	.	.
24/43	23/42 I 1 × 26/42 I 3	53	.	.	3
14/44	22/43 I 3 × 2/43 I 3	66	1	.	5
16/44	24/43 I 1 × 2/43 I 3	89	.	.	4
21/44	21/43 I 9 × 2/43 I 5	44	.	.	3
34/44	11/43 I 19 × 2/43 I 3	11	.	.	.
35/44	11/43 II 1 × 2/43 I 3	26	.	.	.
23/45	14/44 I 6 × 6/44 I 2	112	4	.	4
28/45	19/44 IV 7 × 6/44 I 2	65	1	.	1
29/45	19/44 III 22 × 6/44 I 2	66	2	.	4
30/45	19/44 III 20 × 6/44 I 2	70	2	.	.
31/45	21/44 I 1 × 6/44 I 4	91	1	.	2
32/45	25/44 I 2 × 6/44 I 4	31	.	.	.
33/45	33/44 II 18 × 6/44 I 2	22	1	.	.
34/45	33/44 IV 14 × 6/44 I 2	21	.	.	.
35/45	33/44 VII 12 × 6/44 I 2	65	1	.	6
	Totals	1120	15	2	58

Table 3B. *Progenies of* +P^s/+P^s × *A-Dp* P^r P^s/+P^s

Family	Parents	Progeny			
		+P^s/+P^s	+P^r/+P^s	*A* P^s/+P^s	*A-Dp* P^r P^s/+P^s
15/44	2/43 I 3 × 22/43 I 3	50	.	.	.
17/44	2/43 I 3 × 24/43 I 1	72	.	.	.
22/45*	6/44 I 2 × 14/44 I 6	174 (95 *S*) (71 *s*)	1 (1 *S*)	.	.
	Totals	296	1	.	.

* This family from +P^s/+P^s × *A-Dp* P^r *S* P^s *S*/+P^s *s*; 8 plants failed to flower.

5. The origin of the deficiency and the duplication

The repeated independent occurrence of what is undoubtedly the same deficiency (12 noted, but records probably incomplete) or the same duplication (24 recorded) requires a regular origin by crossing-over. We have seen that the facts can be accounted for if it is supposed that chromosome 3.4 of *O. blandina* has in arm 3 a small non-contiguous direct duplication with the two regions, x_1 and x_2, in one chromosome arm disposed either side of the P^s-*S* region (Fig. 2*a*). If pairing at zygotene in the 3.4 bivalent is occasionally irregular so that the x_1 segment of one chromosome pairs with the x_2 segment of the other and if crossing-over occurs in these abnormally associated segments, the cross-over products would be respectively a duplication and a deficiency. The duplication would have the structure $4.x_1\ P^s\ S\ x_1\ P^s\ S.x_2\ 3$ (Fig. 2*b*) and the deficiency the structure $4.x_2\ 3$ (Fig. 2*c*). The bivalent giving such products would have a characteristic unequal chiasma

(Fig. 3). Such bivalents have not been seen in diploid *O. blandina* and might be difficult of detection if the segment x_1 $P^s S$ were cytologically very short.

The occurrence of the duplication in the normal *blandina* chromosome set has not been observed. The phenotype of a plant heterozygous for such a duplication is a matter for conjecture. Analogy with the duplications recognized suggests it would be little if at all different in appearance from normal *O. blandina*.

Table 4A. *Progenies of A-Dp* P^r S P^s $S/+P^s$ $s \times +P^s$ $s/+P^s$ s

Phenotypes / Family	$+P^s S$	$+P^s s$	$+P^r S$	$+P^r s$	$A\text{-}Dp\ P^{rsr} S$	$A\text{-}Dp\ P^{rsr} S^s$	$A\text{-}Dp\ P^{rsr} s$
23/45	82	30	4	.	1	3	.

TABLE 4B. *Progenies of A-Dp* P^r S P^s $s/+P^s$ $s \times +P^s$ $s/+P^s$ s

Phenotypes / Family	$+P^s S$	$+P^s s$	$+P^r S$	$+P^r s$	$A\text{-}Dp\ P^{rsr} S$	$A\text{-}Dp\ P^{rsr} S^s$	$A\text{-}Dp\ P^{rsr} s$
28/45	(65)		1	.	.	1	.
29/45	(66)		2	.	.	3	1
30/45	(70)		2	.	.	.	.
34/45	(21)		.	.	.	.	.
35/45	(65)		. (1)	.	. (2)	4	.
Totals	(287)		5 (1)	.	. (2)	8	1

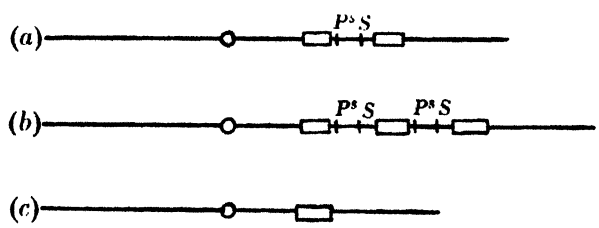

Fig. 2. Inferred structures of chromosome 3.4: (*a*) normal, (*b*) with *P-S* duplication, (*c*) with *P-S* deficiency.

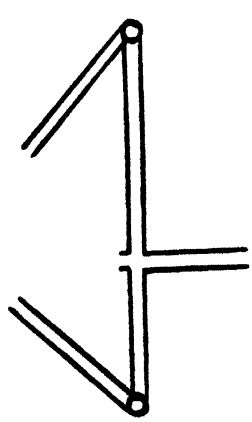

Fig. 3. Type of unequal bivalent expected to follow unequal pairing of duplicate segments.

All the duplications observed have originated in plants heterozygous for the 3.11–4.12 interchange that produces a position effect at the *P* and *S* loci. All the duplications occurred in the 3.11 chromosome of the interchange gamete and the presence of the extra segment was detected by its reaction with the position affected segment contiguous with it.

Actually, when unequal crossing-over occurs in a heterozygous interchange four abnormal products are possible, namely, (1) duplication in interchange 3.11 chromosome, (2) duplication in normal 3.4 chromosome, (3) deficiency in interchange 3.11 chromosome

and (4) deficiency in normal 3.4 chromosome. All except type (2) have been found. Types (2) and (4) should be identical with those arising from unequal crossing over in normal *O. blandina*.

The origin of these four possible types depends upon the fact that two kinds of unequal cross-over figure ought to be possible. These are shown in Fig. 4. Clearly the expected duplications in an interchange chromosome should have the *P-S* segment (P_1 S_1 of Fig. 4) of the original interchange chromosome proximal to the *P-S* segment (P_0 S_0 of Fig. 4) from the original normal chromosome.

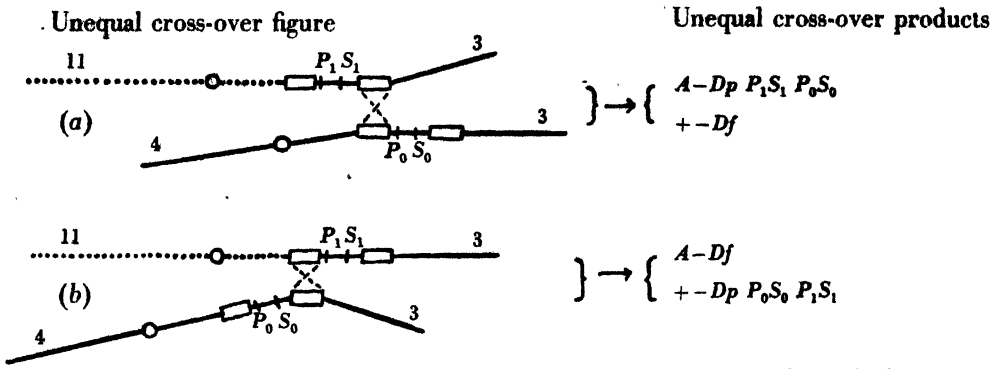

Fig. 4. Types of unequal pairing capable of yielding by crossing-over: (*a*) interchange-duplication and (*b*) interchange-deficiency chromosomes.

Table 5. *Genotypes, phenotypes, cross-over duplication products and origins of duplications in* Oenothera blandina

	Genotype of gamete		Expected phenotype with $+P^s s$	Expected *A-Dp* cross-overs from heterozygote *A-Dp*/ $+P^s s$: Genotypes	Phenotypes	Most likely *A*/+ genotype yielding *A-Dp* chromosome
1	*A-Dp* $P^s S$ $P^s S$	1	*A* $P^s S$	2	II	*A* $P^s S$/ $+P^s S$
2	$P^s S$ $P^s s$	II	$P^s S^e$	—	—	*A* $P^s S$/ $+P^s s$
3	$P^s S$ $P^r S$	III	$P^r S$	4 and 2	IV and II	*A* $P^s S$/ $+P^r S$
4	$P^s S$ $P^r s$	IV	$P^r S^e$	2	II	*A* $P^s S$/ $+P^r s$
5	$P^s s$ $P^s S$	I	$P^s S$	6	V	*A* $P^s s$/ $+P^s S$
6	$P^s s$ $P^s s$	V	$P^s s$	—	—	*A* $P^s s$/ $+P^s s$
7	$P^s s$ $P^r S$	III	$P^r S$	8 and 6	VI and V	*A* $P^s s$/ $+P^r S$
8	$P^s s$ $P^r s$	VI	$P^r s$	6	V	*A* $P^s s$/ $+P^r s$
9	$P^r S$ $P^s S$	VII	$P^{rer} S$	10	VIII	*A* $P^r S$/ $+P^s S$
10	$P^r S$ $P^s s$	VIII	$P^{rer} S^e$	—	—	*A* $P^r S$/ $+P^s s$
11	$P^r S$ $P^r S$	III	$P^r S$	12 and 10	IV and VIII	*A* $P^r S$/ $+P^r S$
12	$P^r S$ $P^r s$	IV	$P^r S^e$	10	VIII	*A* $P^r S$/ $+P^r s$
13	$P^r s$ $P^s S$	VII	$P^{rer} S$	14	IX	*A* $P^r s$/ $+P^s S$
14	$P^r s$ $P^s s$	IX	$P^{rer} s$	—	—	*A* $P^r s$/ $+P^s s$
15	$P^r s$ $P^r S$	III	$P^r S$	16 and 10	VI and VIII	*A* $P^r s$/ $+P^r S$
16	$P^r s$ $P^r s$	VI	$P^r s$	14	IX	*A* $P^r s$/ $+P^r s$

In Table 5 are listed all the sixteen possible interchange chromosome duplications, together with their expected phenotypes (numbers I–IX) when combined with $+P^s s$. It will be noticed that four phenotypes (II, V, VIII and IX) should each have a unique genotype, four others (I, IV, VI and VII) should each have two genotypes, while one phenotype (III) should have four genotypes. In the fifth column of this table are given the genotypes of the most probable interchange heterozygote parents (*A*/+) deduced on the principles enunciated above (Fig. 4). When the observed duplications are tabulated on this basis (Table 6) it is found that nineteen are of the expected composition and only five are exceptional, requiring a more complicated origin that will not be discussed at

present. That the majority are of the expected types constitutes an argument strongly in favour of the above framework of principles.

It seems possible that where a given phenotype includes two or four genotypes, the latter might be distinguishable by means of the kinds of cross-overs arising from an *A-Dp*/ + P^s *s* heterozygote and observed in a backcross to + P^s *s blandina*. Two types of pairing may be expected in such a heterozygote. In one case (Fig. 5*a*) the distal duplicate segment of the interchange 3.11 chromosome pairs with the corresponding region of the normal 3.4 chromosome, in the other case (Fig. 5*b*) the proximal segment of 3.11 is so

Table 6. *Relation between genotype of duplications observed and the interchange heterozygotes that have generated them*

Interchange heterozygote parent	Duplications observed: Expected — Composition	Expected — Phenotype with + P^s *s*	Expected — No. observed	Not expected — Composition	Not expected — Phenotype with + P^s *s*	Not expected — No. observed
A P^s *S*/ + P^r *S*	*A-Dp* P^s *S* P^r *S*	*A* P^r *S*	2	*A-Dp* P^r *S* P^s *S*	*A* P^{rsr} *S*	2
A P^r *S*/ + P^s *s*	*A-Dp* P^r *S* P^s *s*	*A* P^{rsr} S^s	5	*A-Dp* P^r (*S*) P^s *S**	*A* P^{rsr} *S*	1
A P^r *s*/ + P^s *S*	*A-Dp* P^r *s* P^s *S*	*A* P^{rsr} *S*	2	—	—	—
A P^s *S*/ + P^r *s*	*A-Dp* P^s *S* P^r *s*	*A* P^r S^s	9	*A-Dp* P^s *S* P^s *S*	*A* P^s *S*	2
A P^r *S*/ + P^s *S*	*A-Dp* P^r *S* P^s *S*	*A* P^{rsr} *S*	1	—	—	—

* No data are available to show whether the proximal *S*-locus gene was *S* or *s*.

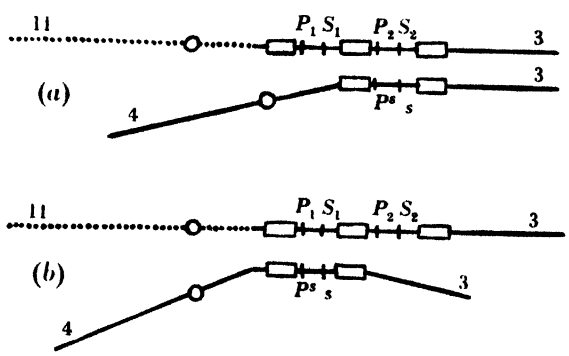

Fig. 5. The two types of pairing possible between an interchange-duplication chromosome and a normal chromosome.

paired. Following crossing-over in the first type the interchange chromosome product would carry the duplication, and would have *s* or P^s and *s* replacing the *P* and *S* locus genes present in the distal part of the duplication. The expectations listed in columns 3 and 4 of Table 5 have been deduced on this basis. Crossing-over in the other type of pairing would yield an interchange chromosome without the duplication and with *s* or P^s and *s* replacing the *P* and *S* locus genes present in the proximal part of the parent duplication. The records suggest that the former type of pairing is by far the commoner one, very few of the cross-overs losing the duplication in the process of their production. The data are, however, not highly critical on the point. The facts are that *A-Dp* P^r P^s/ + P^s gave only 2 *A* P^s chromosomes compared with 58 *A-Dp* P^r P^s chromosomes, while *A-Dp* P^s P^r/ + P^s gave 18 *A-Dp* P^s P^r compared with 10 *A* P^s or *A-Dp* P^s P^s chromosomes (of which probably a majority carried the duplication). It appears that crossing-over between the *P-S* region of a normal chromosome and the distal *P-S* region of an *A-Dp* chromosome is exceptionally frequent. This is also demonstrated by the structurally

homozygous progeny (§ 7). The behaviour supports the view that in *Oenothera* pairing normally commences at the ends of the chromosomes and progresses towards the centromere.

6. Position effect in the duplication

The genes P^s or P^r and S when adjacent to the interchange break are subject to a position effect of the variegation type. These genes show the same behaviour when they are in the interchange-duplication (*A-Dp*) chromosome, but only if they are in the proximal segment of the duplication. When the genes are in the distal segment of the duplication they show no variegation. This is demonstrated by *A-Dp* P^s $P^r/+P^s$ plants which have entirely deep red sepals whereas *A-Dp* P^s $P^s/+P^s$ plants have regular medium red striped buds, and by *A-Dp* s $S/+s$ plants which have wholly yellow petals while *A-Dp* s $s/+s$ plants have sulphur petals. An *A-Dp* P^r $P^s/+P^s$ plant has sepals with patches of deep red disposed like the red patches of A $P^r/+P^s$. The intervening areas are uniformly a medium red such as would be expected if the P^s genes were uniformly pigmenting the sepals as in $+P^s/+P^s$ and not showing a variegation as in A $P^s/+P^s$, with green tissue intervening between the blocks of medium red. If both P^r and P^s in the *A-Dp* chromosome were showing variegation one would expect to see at least some small well-defined green areas where the deep red of P^r variegation and the medium red of P^s variegation were both absent.

Thus there is a difference in position effect of the same genes in the two sections of the *A-Dp* chromosome. Whatever determines the variegation position effect, presumed to be heterochromatinization sometimes inhibiting the gene, it is evident that it does not spread beyond the end of the proximal segment. Further it is improbable that the x_1 and x_2 segments are heterochromatic, otherwise one would expect that the P and S genes within the distal segment of the *A-Dp* chromosome would also show variegation. The duplication has therefore rather little significance for the interpretation of the position effect. It does show that the effect cannot depend upon the non-contiguous duplicate segments of the A chromosome and confirms the belief that the position effect depends solely on the genes being adjacent to heterochromatin.

7. Crossing-over in the duplication

A detailed consideration of the various duplication genotypes and their progenies is hardly profitable at the present time since rather few critical data have been obtained. It is possible that no less than eight of the gametic types (numbers 1, 2, 3, 4, 9, 10, 13 and 14) have occurred in the cultures, but experiments have been confined to just a few of them (especially numbers 3, 9 and 10). The available evidence shows that crossing-over in a duplication heterozygote is very peculiar, partly because it mainly involves the distal segment of the duplication (see § 4) and partly because crossing-over is very free giving recombination fractions apparently in excess of 50%.

Tables 7 A and 7 B incorporate the data available from the backcrosses

$$A\text{-}Dp\ P^s\ P^r/+P^s \times +P^s/+P^s \quad \text{and} \quad A\text{-}Dp\ P^s\ S\ P^r\ s/+P^s\ s \times +P^s\ s/+P^s\ s.$$

Confining attention to the normal, non-interchange and presumably non-duplication progeny there are seventy-eight cross-overs between P^r and the interchange locus out of a total of 166 plants, i.e. 47% recombination. Further, there are fourteen cross-overs between S and the interchange locus in a total of 127 plants, i.e. 11% recombination. The latter, most surprisingly, is about the same as the 8·5% found in A $S/+s$ heterozygotes.

In duplications of the type *A-Dp* P^r $P^s/+P^s$ (Table 3A), 15 cross-overs between P^r and the locus of the interchange were found in a total of 1135 plants, i.e. 1·3% recombination, again a value close to the 1·75% found in *A* $P^r/+P^s$ plants. In one family (Table 4A) there were 86 cross-overs between the distal *S* locus and the interchange locus in a total of 116 plants, i.e. 74% recombination. A reciprocal (Table 3B) showed 96 cross-overs

Table 7A. *Progenies of A-Dp* P^s $P^r/+P^s \times +P^s/+P^s$

Family	Parents	Progeny $+P^s/+P^s$	$+P^r/+P^s$	*A* $P^s/+P^s$*	*A-Dp* P^s $P^r/+P^s$
1/41	69/40 II 12 self	5	19	1	.
2/41	69/40 II 12 × 1/40 I 2	6	12	.	1
36/44	12/43 III 38 × 2/43 I 3	9	2	.	1
37/44	13/43 III 1 × 2/43 I 5	15	11	.	2
38/44	13/43 III 35 × 2/43 I 3	6	6	.	5
39/44	13/43 IV 2 × 2/43 I 5	20	12	4	1
41/44	14/43 IV 13 × 2/43 I 5	4	5	.	1
43/44	15/43 V 7 × 2/43 I 3	28	30	6	7
	Totals	88	78	10	18

* Some or all these plants may be *A-Dp* P^s $P^s/+P^s$.

Table 7B. *Progenies of A-Dp* P^s *S* P^r $s/+P^s$ $s \times +P^s$ $s/+P^s$ *s*

Phenotype / Family	$+P^s$ *S*		$+P^s$ *s*	$+P^r$ *S*		$+P^r$ *s*	*A-Dp* (?) P^s S^e	*A-Dp* P^r S^e
36/44	0	(1)*	8	.		2	.	1
37/44	1	(2)	12	.		11	.	2
38/44	2		4	1		5	.	5
39/44	3		17	2		10	4	1
41/44	.		4	3		2	.	1
43/44	2	(9)	17	.	(9)	21	3 (3)	6 (1)
Totals	8	(12)	62	6	(9)	51	7 (3)	16 (1)

* Numbers in brackets are of plants that failed to flower and so could not be scored for *S*.

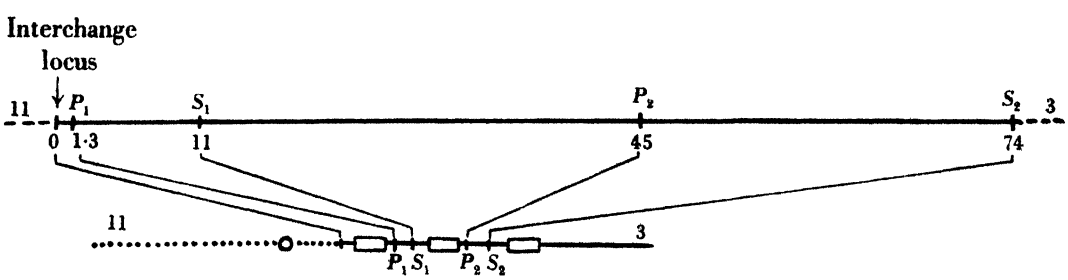

Fig. 6. Preliminary genetical and cytological maps of the interchange-duplication chromosome.

amongst 167 plants, i.e. 57·5% recombination. These are undoubtedly excessive estimates due to *s* gametes being less viable than *S* gametes. Material in which the viability effect can be estimated is not yet available, so the further consideration of the linkage map of the duplication (Fig. 6) and of the possibility of recombination exceeding 50% must be deferred. However it does appear that there are local changes in ease of crossing-over along the chromosome.

8. Summary

A direct contiguous duplication of the P^s-*S* region of chromosome arm 3 in *Oenothera blandina* has been found genetically in the interchange chromosome 3.11. Twenty-four separate occurrences of it in the progeny of the interchange heterozygote have been

observed and indicate that the duplication must arise by unequal crossing-over. A corresponding deficiency of the P^s-S region from chromosomes 3.4 and 3.11 has also been found. Crossing-over within the duplication is surprisingly frequent and exhibits various anomalies.

REFERENCES

Bridges, C. B. (1935). *Biol. Zh.* **4**, 401–20.

Bridges, C. B. (1936). *Science*, **83**, 210–11.

Bridges, C. B. & Brehme, K. S. (1944). The mutants of *Drosophila melanogaster*. *Publ. Carneg. Instn*, no. 552.

Catcheside, D. G. (1932). *Cytologia, Tokyo*, **4**, 68–113.

Catcheside, D. G. (1946). *J. Genet.* **48**, 31–42.

Darlington, C. D. (1930). *Cytologia, Tokyo*, **2**, 37–55.

Demerec, M. & Hoover, M. E. (1939). *Genetics*, **24**, 68.

Dobzhansky, Th. (1934). *Z. indukt. Abstamm.- u. VererbLehre*, **68**, 143.

Lewis, E. B. (1945). *Genetics*, **30**, 137–66.

Morgan, L. V. (1938). *Genetics*, **23**, 423–62.

Schultz, J. (1941). *D.I.S.* **14**, 54–5.

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CHIASMA INTERFERENCE IN MOSQUITOES

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(With Five Text-figures)

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1. INTRODUCTION

Muller (1916) first demonstrated interference between cross-overs in *Drosophila*. In 1931 Haldane, by consideration of the variance of chiasma frequency data from a number of plants, showed that interference is also a property of chiasmata. The correspondence between these two discoveries is one among the many arguments for the identity of cross-overs and chiasmata.

Abundant evidence is now to hand which proves the existence of cross-over or chiasma interference within the chromosome arm on one side of the centromere. However, when both chromosome arms separated by the centromere are considered together, most of the evidence, both genetical and cytological, demonstrates independence of the two (Mather, 1936*b*). Two geneticists (Graubard, 1932; Schweitzer, 1935) claimed to have shown positive interference across the centromere in chromosome II of *D. melanogaster*, but Stevens (1936) has shown that their conclusions were fallacious, having been based on inconsistent measures of coincidence.

Positive correlation, i.e. negative interference, between cross-overs in regions near to the centromere has been observed in a number of genetical experiments concerned with the IIIrd chromosome of *D. melanogaster* (summarized by Kikkawa, 1935). Newcombe (1941) analysed the data of Lindegren & Lindegren (1937) from *Neurospora* and found similar positive correlation of cross-overs on either side of the centromere in this fungus.

Pätau (1941) has drawn attention, however, to a result obtained by Gowen (1919) in a study of crossing-over in chromosome III of *Drosophila melanogaster*. Pätau's calculations of coincidence by Stevens's method and based on Gowen's data show that whereas between the sections *se-D* and *ss-e*, which are separated by a region containing the centromere, interference is negative, between *se-D* and *e-ro*, which are separated by a larger region containing the centromere, interference is positive. This appears to be the

only example from genetical experiment of positive cross-over interference developed across the centromere.

The evidence from cytology has been in line with that from genetics. Thus Bennett (1938) was able to show independence of the chromosome arms in *Fritillaria chitralensis*, and Barber (1941) found the same condition to hold for *Uvularia perfoliata*. If, on the other hand, any correlation in the formation of chiasmata on either side of the centromere were demonstrable, the correlation was positive (Callan, 1940, in *Anilocra*; Newcombe, 1941, in *Trillium*).

Pätau (1941) has now clearly demonstrated that the opposite condition, i.e. negative correlation between chiasmata formed on either side of the centromere, occurs in two species of the Diptera Nematocera. He states in the summary of his paper that 'bei *Culex pipiens* wahrscheinlich und bei *Dicranomyia trinotata* mit sehr hoher statistischer Sicherung positive Interferenz über das Centromer existiert'. We have been able to confirm and extend Pätau's conclusion as regards chiasma interference across the centromere in *Culex*.

2. Material and technique

Larvae and pupae of two species of mosquito were collected from stagnant fresh water at the Stazione Zoologica, Naples, during the summer months of 1945. The two species were identified as *Culex pipiens* L. and *Theobaldia* (*Allotheobaldia*) *longiareolata* Macq.

The testes from late larval or pupal stages were dissected out under a binocular microscope, transferred for a few moments to a fixative consisting of three parts absolute alcohol: one part glacial acetic acid, and then stained in bulk on a slide in iron aceto-carmine. After staining for about 5 min., the testes were squashed out lightly under a coverslip and examined direct. In the case of *Theobaldia* the preparations were made permanent according to the method of La Cour (1937). With *Culex* this was not successful, however, since much of the material failed to stick either to coverslip or slide at separation, and contraction and distortion occurred in that portion remaining attached. *Culex* material was thus examined fresh and thrown away after use. This unfortunate characteristic of the neapolitan *Culex* was not present in *C. pipiens* obtained from a London source and examined by one of us in 1940.

3. *Culex pipiens* L. Chiasma interference within the bivalent

The diploid complement of *Culex* consists of six chromosomes, all of which possess median centromeres (Stevens, 1910; Moffett, 1936). Two of the chromosome pairs (*M*) are of equal length, whilst the third pair (*m*) is somewhat shorter, the length relationship between them at mitotic metaphase being approximately as three to two. Two spermatogonial metaphases and three second meiotic metaphases are shown in Figs. 1 and 2. The somatic pairing characteristic of dipteran cells was observed in all mitoses. No sex chromosomes are distinguishable.

Six typical first meiotic metaphases are shown in Fig. 3. Evidently the rule is for no chiasma or one chiasma to be formed within a single arm pair. Very occasionally two chiasmata are formed within one arm pair: the frequency of this occurrence varies from individual to individual, the average being 1·74% of the total bivalents observed. There is perhaps a tendency in scoring to exaggerate the numbers of such exceptional bivalents: in normal bivalents the chiasma counts can be relied on as entirely accurate, the con-

figurations being simple. Where doubt has arisen over the scoring of two chiasmata in an arm pair, these have always been scored as two. In calculations of chiasma interference within the bivalents these exceptional bivalents have in all cases been neglected.

The mean chiasma frequencies per cell (neglecting those cells containing exceptional bivalents) for the ten individuals examined are listed in Table 1, the individuals being arranged in ascending order of chiasma frequency. The group is not homogeneous for this

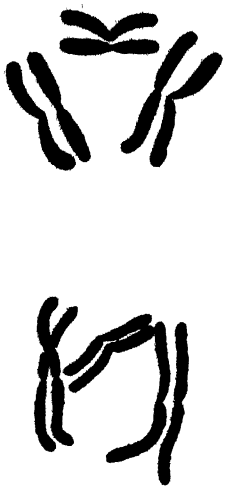

Fig. 1. *Culex pipiens*. Two spermatogonial mitoses. ×3500.

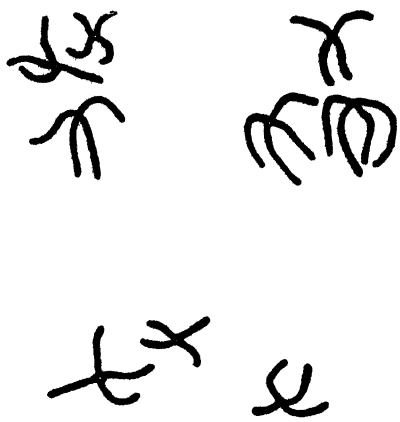

Fig. 2. *Culex pipiens*. Three secondary spermatocyte divisions. ×3500.

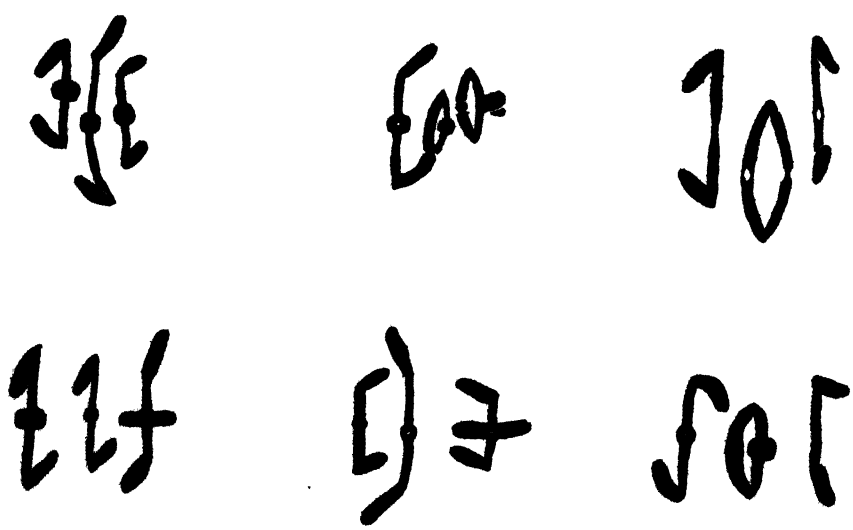

Fig. 3. *Culex pipiens*. Six primary spermatocyte divisions. ×3500.

character. The general contingency Table 2 (Mather, 1943*b*, p. 200) records the number of cells with various chiasma frequencies from two to six in the ten specimens. On the basis of the marginal totals, figures of expectation have been calculated assuming the data to be homogeneous. In applying the χ^2 test of agreement between observed and expected figures, the two and three chiasmata and five and six chiasmata classes have been pooled because of the low figures of expectation in the two and six chiasmata classes.

The total χ^2 of 57·889, with 18 degrees of freedom, indicates that the chance of this divergence from expectation being due to sampling errors is very small (at $P=0{\cdot}001$, $n=18$, $\chi^2=42{\cdot}31$).

Table 1. *Culex pipiens*

Specimen no.	No. of cells observed	Mean chiasma frequency per cell	Standard error ±	Metaphase terminalization coefficient	Exceptional bivalents %	Index of interference based on '*M*'+'*m*' data	Index of interference based on '*M*' data alone
1	36	3·139	0·107	0·556	0·90	0·808	0·827
9	92	3·500	0·070	0·395	1·05	0·675	0·648
10	111	3·523	0·064	0·557	1·16	0·671	0·712
6	46	3·587	0·101	0·500	3·27	0·529	0·414
8	37	3·595	0·106	0·511	0·00	0·669	0·626
7	38	3·763	0·116	0·548	2·44	0·600	0·534
4	151	3·821	0·064	0·547	1·48	0·577	0·594
2	71	3·873	0·104	0·601	1·78	0·447	0·426
3	53	4·038	0·132	0·533	3·39	0·457	0·476
5	19	4·105	0·202	0·579	4·54	0·465	0·414

Table 2. *Culex pipiens*

(Figures of expectation are given in brackets.)

Specimen no.	No. of chiasmata per cell: 2	3	4	5	6	Total cells
1	5	21	10	0	0	36
	(0·7156)	(15·5780)	(14·7523)	(4·1835)	(0·7706)	
9	1	51	34	5	1	92
	(1·8287)	(39·8105)	(37·7004)	(10·6911)	(1·9694)	
10	2	58	42	9	0	111
	(2·2064)	(48·0321)	(45·4863)	(12·8991)	(2·3762)	
6	2	18	23	3	0	46
	(0·9144)	(19·9051)	(18·8500)	(5·3455)	(0·9847)	
8	0	18	16	3	0	37
	(0·7355)	(16·0107)	(15·1621)	(4·2997)	(0·7921)	
7	0	14	20	3	1	38
	(0·7554)	(16·4434)	(15·5719)	(4·4159)	(0·8135)	
4	0	59	64	24	4	151
	(3·0016)	(65·3419)	(61·8785)	(17·5476)	(3·2325)	
2	1	25	31	10	4	71
	(1·4113)	(30·7225)	(29·7187)	(8·2506)	(1·5198)	
3	2	14	20	14	3	53
	(1·0535)	(22·9343)	(21·7187)	(6·1590)	(1·1346)	
5	0	5	8	5	1	19
	(0·3777)	(8·2217)	(7·7859)	(2·2079)	(0·4067)	
Total cells	13	283	268	76	14	654

Table 3. *Culex pipiens*

Specimen no.		Mean chiasma frequency per cell	Standard error ±	Terminalization coefficient
4	Diplotene	4·064	0·134	0·533
	Metaphase	3·712	0·069	0·547
9	Diplotene	3·556	0·105	0·317
	Metaphase	3·421	0·081	0·395
10	Diplotene	3·476	0·092	0·395
	Metaphase	3·536	0·087	0·557

In specimens 4, 9 and 10 two separate sets of chiasma counts were made for cells in diplotene or diakinesis and for cells at metaphase. Table 3 sets out the mean chiasma frequencies and their standard errors, as observed both before and at metaphase in these three individuals, together with the terminalization coefficients (Gairdner & Darlington, 1931) at the two stages. In specimens 9 and 10 the chiasma frequency differences are not

significant. The difference figure for specimen 4 is on the borderline of significance: however, this is certainly due to our tendency at this stage of the investigation to overcount the number of 'rings' at diplotene. In many bivalents where only one chiasma has occurred there is a stage when the free arms temporarily remain touching at their extremities before pulling entirely apart due to the activation of the centromeres. We were later able to distinguish between this condition and that of a true fully terminalized chiasma: in the former the arm pairs near to the point where they touch lie close together and parallel to one another, whereas in the latter the arm pairs lie extended in line through the terminalized chiasma. It is significant that the higher diplotene chiasma frequency of specimen 4 is not due to an excess in the proportion of bivalents with more than one chiasma in an arm pair: moreover, it would be the first observation of its kind were it found that fully terminalized chiasmata sometimes break their connexion during the passage from diplotene to metaphase. In short we feel confident that in *Culex* the chiasmata visible at metaphase are not less in number than those which originally formed at pachytene.

In all three specimens the terminalization coefficient at metaphase is somewhat higher than that at diplotene. Moffett made a similar observation, and he thus assumed that a slight movement of interstitial chiasmata towards the distal ends of the chromosomes took place between diplotene and metaphase. However, the differences which we have observed are so small that they might equally well be ascribed to the greater ease of resolution between subterminal and terminal chiasmata at diplotene as compared with metaphase.

(*a*) *The evidence from chiasma number*

For the purpose of argument, let us assume that each chromosome arm of *Culex* has an equal chance of forming a chiasma and also that the two arms of a bivalent separated by the centromere act independently of one another. The chiasma statistics then provide the information necessary for the calculation of a theoretical figure, p, the chance that one arm pair has of forming one chiasma. $1-p$, or q, is thus the chance of a chiasma failing to form in one arm pair. Then p^2 is the expectation of the formation of two chiasmata, one in each arm, $2pq$ is the expectation of the formation of only one chiasma, and q^2 is the expectation of chiasma failure in both arms together. In specimen 1, for example, ten bivalents were observed to have formed two chiasmata each (20 chiasmata), ninety-five bivalents formed one chiasma each (95 chiasmata) and five pairs of univalents failed to form chiasmata. A total of 115 chiasmata were thus formed by 110 bivalents, i.e. between 220 arm pairs. p is thus $115/220 = 0{\cdot}5227$ and $q = 0{\cdot}4773$. Table 4 shows the expected and observed proportions (in percentages) of two-chiasmata bivalents, one-chiasma bivalents and univalent pairs in the ten specimens examined. In all of these there is a consistent divergence of the observed from the expected proportions, so clear that a test of significance is unnecessary. The observed proportions of single-chiasma bivalents are always in excess of expectation, whilst univalent pairs and two-chiasmata bivalents show a deficit.

If, therefore, we make the initial assumption that in any one individual of *Culex* each chromosome arm has the same intrinsic chance of forming a chiasma, then it is clear that the arms are not independent of one another and, moreover, the chiasma correlation in the two arms is negative, i.e. there is positive chiasma interference developed across the

centromere. The specimens differ in their degree of divergence from expectation, and it is necessary to arrive at a measure of interference in order to compare one with another.

Suppose that a = number of bivalents with two chiasmata, $2b$ = number of bivalents with one chiasma and c = number of univalent pairs, then the chance of formation of a chiasma in the left (or right) arm in the absence of one in the other is $p_1 = \frac{b}{b+c}$, and the chance of formation of a chiasma in the left (or right) arm in the presence of one in the other is $p_2 = \frac{a}{a+b}$. We have chosen as *index of interference* the complement of the ratio $\frac{p_2}{p_1}$ i.e. $1 - \frac{a(b+c)}{b(a+b)}$.* When there is no interference, $p_1 = p_2$ and the index of interference = 0.

Table 4. *Culex pipiens*

(Figures of expectation are given in brackets.)

Specimen no.	Percentages of bivalent types		
	X/X	*X/O*	*O/O*
1	9·09	86·36	4·55
	(27·32)	(49·90)	(22·78)
2	32·58	64·25	3·17
	(41·86)	(45·68)	(12·46)
3	35·08	63·74	1·17
	(44·84)	(44·25)	(10·92)
4	26·55	73·23	0·21
	(39·90)	(46·53)	(13·56)
5	36·51	63·49	0·00
	(46·58)	(43·34)	(10·08)
6	22·97	70·27	6·76
	(33·76)	(48·69)	(17·56)
7	25·00	75·00	0·00
	(39·06)	(46·88)	(14·06)
8	19·81	80·18	0·00
	(35·88)	(48·02)	(16·08)
9	18·08	80·14	1·77
	(33·82)	(48·67)	(17·50)
10	18·77	80·06	1·17
	(34·57)	(48·45)	(16·97)

When interference is complete, $p_2 = 0$ and the index of interference = 1. The indices of interference for the ten individuals are listed in Table 1.

In general the '*m*' chromosomes form fewer two-chiasmata bivalents than do the '*M*' chromosomes. We attempted in all cells to distinguish between '*M*' and '*m*' bivalents. While this is possible in most cases there is a certain initial tendency to exaggerate the number of two-chiasmata '*m*' bivalents since such associations often give the general impression of being smaller than those which have formed only one chiasma.

Our deductions as to interference can thus be criticized on the grounds that we make the unjustifiable assumption that the arm pairs of the '*M*' and '*m*' bivalents have equal chances of chiasma formation. The criticism is valid: however, further reference to Table 1 will show that the indices of interference, whether calculated on pooled '*M*' and '*m*' data, or based on '*M*' data alone, are in all individual cases considerably less than unity: moreover, except in the case of specimen 6, the difference between the two indices calculated for each individual is always less than 0·1unit. In view of the fact that the '*m*'

* Pätau (1941) has used two measures of interference, neither of which is identical with the one arrived at above. Provided the measure is logically derived, however, the precise form of its employment is a matter of convenience only.

bivalents are often difficult to distinguish from the '*M*' bivalents, we would place more reliance on the indices calculated on the mixed statistics.

Table 1 shows that mean chiasma frequency per cell and index of interference are inversely correlated, as, indeed, they must be provided univalents are rare. Specimen 6 was abnormal in showing 6·8% univalents (and a higher proportion of these in the '*M*' than in the '*m*' bivalents, which is also exceptional). There was, furthermore, extensive breakdown of the spindle at first meiotic metaphase in this specimen.

We must, however, now return to consider the initial assumption on which our deduction and analysis of interference is based. Altogether apart from the question of differences between '*M*' and '*m*' bivalents, we have assumed that cytologically indistinguishable arms separated by the centromere have equal intrinsic chances of taking part in a chiasma. If, on the other hand, one assumes that the arms are independent of one another in the formation of chiasmata, i.e. there is no interference across the centromere, then it is possible to calculate different chances of chiasma formation in the two arms which could give rise to the observed proportions of the various bivalent types. The calculation also involves the assumption that each chromosome of the complement contributes the same inequality.

If a, $2b$ and c are the numbers of two-chiasmata bivalents, one-chiasma bivalents and univalent pairs respectively, then it can be shown that the 'high' and 'low' chances of chiasma formation (p) are given by the two roots of the quadratic equation

$$(a+2b+c)\,p^2-(2a+2b)\,p+a=0,$$

$$p=\frac{a+b\pm\sqrt{(b^2-ac)}}{a+2b+c}.$$

Thus in the case of specimen 1, where $a=10$, $2b=95$ and $c=5$, the 'high' chance $=0{\cdot}9497$ and the 'low' chance $=0{\cdot}0957$. The ratio of 'high' to 'low' is 9·92, i.e. one arm has roughly ten times greater prospect of forming a chiasma than has the other. The chance ratios range from 3/1 to 10/1 in the ten specimens examined.

Intrinsic inequalities of chance of these orders of magnitude seem most improbable unless in *Culex* we are dealing with structural heterozygosity of a type and on a scale previously undescribed. Since we have not seen a single bridge and fragment at meiotic anaphases, such a postulate appears unjustified. On the basis of this argument we are thus led to the same conclusion as that arrived at by Pätau: minor intrinsic inequalities of chance of chiasma formation may well exist between the chromosome arms of *Culex*; however, chiasma interference across the centromere is probably the main factor which determines the disproportionately large number of chromosomes which have formed chiasmata on one side of the centromere alone.

(*b*) *The evidence from chiasma position*

We have now been able to carry the analysis a step further, thereby clinching the argument. In scoring the chiasmata of *C. pipiens* we scored as '*P*' those which occurred within the proximal half of the arm pair and as '*D*' those in the distal half. The letter '*O*' indicates the absence of a chiasma. Naturally great observational errors are involved, and in any case the absolute figures may have little meaning if there is chiasma movement in this species. The relative proportions of *O/P*, *O/D*, *P/P*, *P/D* and *D/D* bivalents, however, throw light on the interference question.

Specimens 2, 4, 9 and 10 were selected as being those where most cells had been analysed. The proportions in which the various kinds of '*M*' bivalents were observed are listed in Table 5. Also listed are the proportions to be expected on the assumptions that the two arm pairs of each bivalent have equal pairing chances and behave independently of one another. Both observed and expected percentages were adjusted to omit the figures for *O/O* bivalents (i.e. univalents). In Table 6 the ratios of observed to expected percentages of all the various types of bivalent in the four individuals are listed side by side. Single-chiasma bivalents are, as we already know, in excess of expectation, the excess being greater in the case of *O/P* than in that of *O/D* bivalents. This indicates that a proximal chiasma in one arm is more effective than a distal in suppressing a chiasma in the other arm. Two-chiasmata bivalents show a deficit on expectation: the divergence is greatest in the case of *P/P* bivalents, least in that of *D/D* bivalents, with *P/D* bivalents falling between.

The figures for both one-chiasma and two-chiasmata bivalents thus agree in showing that the power of one chiasma to suppress the formation of another falls off with distance

Table 5. *Culex pipiens*

(Figures of expectation are given in brackets.)

Specimen no.	Percentages of bivalent types				
	O/P	*O/D*	*P/P*	*P/D*	*D/D*
2	13·02	47·95	0·68	10·95	27·39
	(8·62)	(38·64)	(1·75)	(15·73)	(35·26)
4	26·45	48·06	0·32	8·39	16·77
	(15·35)	(38·93)	(3·66)	(18·54)	(23·51)
9	21·92	56·68	0·00	6·42	14·97
	(13·18)	(43·25)	(2·38)	(15·59)	(25·60)
10	20·35	56·64	0·44	3·54	19·03
	(11·20)	(44·38)	(1·80)	(14·31)	(28·32)

Table 6. *Culex pipiens*

Specimen no.	Ratio of observed/expected frequencies of bivalent types				
	O/P	*O/D*	*P/P*	*P/D*	*D/D*
2	1·510	1·241	0·388	0·696	0·777
4	1·723	1·234	0·087	0·453	0·713
9	1·663	1·311	0·000	0·412	0·585
10	1·817	1·276	0·244	0·247	0·672

across the centromere, just as it does in the more generally known phenomenon where interference is said to be confined within one arm on one side of the centromere.

The data regarding chiasma position can be analysed in another way to demonstrate more precisely the relationship between interference and distance along the chromosome. Thus the proportions of *O/O*, *O/P* and *O/D* bivalents provide the information necessary for the calculation of the chances of formation of *O*, *P* or *D* chiasmata in one arm given no chiasma in the other. If *OO*, *OP* and *OD* stand for the numbers of *O/O*, *O/P* and *O/D* bivalents respectively, then, given no chiasma in one arm,

$$\text{the chance of formation of } O \text{ in the other} = \frac{OO}{OO+\frac{1}{2}OP+\frac{1}{2}OD},$$

$$\text{the chance of formation of } P \text{ in the other} = \frac{\frac{1}{2}OP}{OO+\frac{1}{2}OP+\frac{1}{2}OD},$$

$$\text{the chance of formation of } D \text{ in the other} = \frac{\frac{1}{2}OD}{OO+\frac{1}{2}OP+\frac{1}{2}OD}.$$

Similarly, if O/P, P/D and P/P are considered together, then, given a proximal chiasma in one arm,

$$\text{the chance of formation of } O \text{ in the other} = \frac{\frac{1}{2}OP}{PP+\frac{1}{2}OP+\frac{1}{2}PD},$$

$$\text{the chance of formation of } P \text{ in the other} = \frac{PP}{PP+\frac{1}{2}OP+\frac{1}{2}PD},$$

$$\text{the chance of formation of } D \text{ in the other} = \frac{\frac{1}{2}PD}{PP+\frac{1}{2}OP+\frac{1}{2}PD}.$$

Finally, if O/D, P/D and D/D are considered together, then, given a distal chiasma in one arm,

$$\text{the chance of formation of } O \text{ in the other} = \frac{\frac{1}{2}OD}{DD+\frac{1}{2}OD+\frac{1}{2}PD},$$

$$\text{the chance of formation of } P \text{ in the other} = \frac{\frac{1}{2}PD}{DD+\frac{1}{2}OD+\frac{1}{2}PD},$$

$$\text{the chance of formation of } D \text{ in the other} = \frac{DD}{DD+\frac{1}{2}OD+\frac{1}{2}PD}.$$

Table 7 shows the various figures of chance, expressed as percentages, for specimens 2, 4, 9 and 10. Taking specimen 2 as an example and reading the columns vertically, we see

Table 7. *Culex pipiens*

		Specimen no.	Chance of chiasma in other arm		
			O	*P*	*D*
Given initial chiasma in one arm	*O*	2	2·198	20·879	76·923
		4	0·000	35·498	64·502
		9	0·000	27·891	72·109
		10	0·000	26·437	73·563
	P	2	51·351	5·405	43·243
		4	74·546	1·818	23·636
		9	77·359	0·000	22·642
		10	82·143	3·571	14·286
	D	2	42·168	9·639	48·193
		4	53·405	9·319	37·276
		9	60·920	6·897	32·184
		10	57·658	3·604	38·739

Table 8. *Culex pipiens*

		Specimen no.	Chance of chiasma formation in one arm in the presence of a chiasma in the other arm divided by Chance of chiasma formation in one arm in the absence of a chiasma in the other arm	
			P	*D*
Given initial chiasma in one arm	*P*	2	0·259	0·562
		4	0·051	0·366
		10	0·135	0·194
		9	0·000	0·314
	D	2	0·462	0·627
		4	0·262	0·578
		10	0·136	0·526
		9	0·247	0·446

that the chance of formation of a proximal chiasma in one arm falls from 20·9:9·6:5·4 according as to whether the other arm has formed no chiasma or a distal or a proximal chiasma. The figures in the various cells of this table are reasonably consistent from

specimen to specimen, thus all show the same trends. The reduction in the chance of formation of '*P*' or '*D*' chiasmata in one arm imposed by '*P*' or '*D*' chiasmata in the other arm, expressed as ratios of the chances in the absence of a chiasma in the other arm, are measures of interference. They are listed in Table 8. The specimens have been arranged in order of index of interference as computed from the data of chiasma number alone. The seriation of the chance ratios is in tolerably good agreement with this order.

All the evidence thus converges on the view that in *C. pipiens* chiasma number and position are largely determined by chiasma interference, against which the centromere is no barrier.

4. *Theobaldia* (*Allotheobaldia*) *longiareolata* Macq.

The diploid complement of *Theobaldia*, like that of *Culex*, consists of six chromosomes. Two pairs of larger chromosomes are of equal overall length: one of these has median centromeres while in the other the centromeres are very slightly off-median. The third

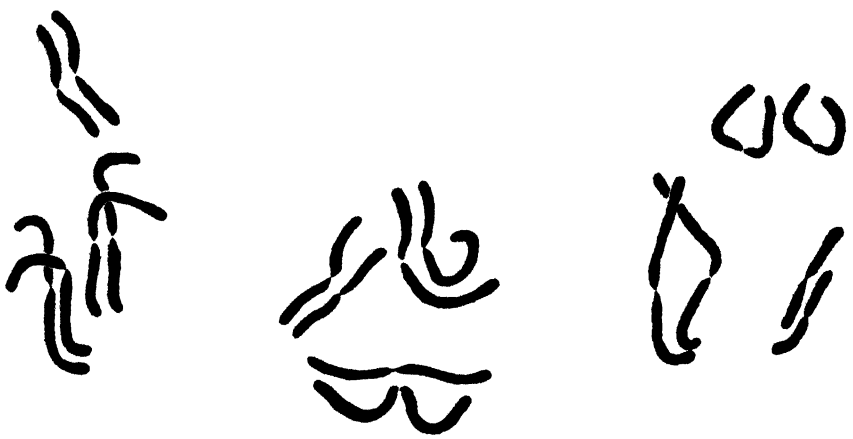

Fig. 4. *Theobaldia longiareolata*. Three oogonial mitoses. × 3500.

Fig. 5. *Theobaldia longiareolata*. Two primary spermatocyte divisions. × 3500.

chromosome pair has median centromeres and is shorter than the other pairs, the length relationship between them being approximately as three to two. As in the description of *Culex*, the larger chromosomes will be referred to as '*M*' and the smaller as '*m*'. Three oogonial mitoses are reproduced in Fig. 4. In Fig. 5 two first meiotic metaphases are shown. Their appearance contrasts markedly with that of the similar stage in *Culex*, since the '*M*' bivalents of *Theobaldia* often form two chiasmata and sometimes three within the arm pair.

Theobaldia is not so suitable for cytological study as is *Culex*. The configurations of the bivalents are often complex, and unfortunately only a dozen or so cells are to be found at

first meiotic metaphase in any one individual, in contrast to the hundreds of such stages often available in *Culex*.

Table 9 lists the mean chiasma frequency per cell, its standard error and the terminalization coefficient for six individuals. The means at the two extremes of the scale are not significantly different from one another. Thus in order to compare the chiasma statistics of *Theobaldia* with those of *Culex*, the figures for all six individuals have been pooled.

The '*M*' bivalents have a mean chiasma frequency of 2·911, standard error ±0·069, whereas the '*m*' bivalents have a mean chiasma frequency of 1·953, standard error ±0·041. In contrast to the '*M*' bivalents, the '*m*' bivalents rarely form more than one chiasma within the arm pair.

Table 9. *Theobaldia longiareolata*

Specimen no.	No. of cells observed	Mean chiasma frequency per cell	Standard error	Terminalization coefficient
1	16	7·438	0·418	0·235
4	9	7·444	0·412	0·254
6	16	7·500	0·408	—
2	9	8·000	0·372	0·208
3	14	8·143	0·433	0·166
5	14	8·143	0·455	0·158

Table 10. *Theobaldia longiareolata*

Bivalent type	Observed	Expected
O/O	0	0·07
O/X	5	3·69
O/XX	2	2·96
O/XXX	0	0·20
X/X	51	47·12
X/XX	69	75·80
X/XXX	3	5·25
XX/XX	33	30·48
XX/XXX	7	4·23
XXX/XXX	0	0·15
Total	170	

Taken separately, neither the '*M*' nor the '*m*' bivalent statistics provide any evidence of chiasma interference across the centromere. The '*m*' bivalents form simple 'rings' almost without exception. The observed and expected figures of the various types of '*M*' bivalent association, considered without regard for chiasma position, are listed in Table 10. The figures of expectation are based on the assumptions that the arm pairs have equal intrinsic chances of forming chiasmata and that they behave independently of one another. There is good agreement between observed and expected figures ($\chi^2 \doteq 1{\cdot}148$, $n=3$, $P>0{\cdot}7$).

Unfortunately, the number of cells of *Theobaldia* which were studied is insufficient to provide a comprehensive test of the distribution of chiasma position, but the figures available give no indication of negative correlation between chiasmata on either side of the centromere; in fact, though the deviation is not statistically significant, *P/P* bivalents are in excess of expectation.

5. DISCUSSION

In *Culex* we have clear evidence of chiasma interference across the centromere, whereas in *Theobaldia* there appears to be none. The *Theobaldia* data are admittedly few and incompletely analysed, but we feel sure that even were they more extensive, treatment by

the method applied to *Culex* would fail to give evidence of chiasma interference across the centromere. None of the bivalents of *Theobaldia* have chromosome arms which are distinguishable from one another at meiosis: were it otherwise, a more extensive investigation would have been justified.

Culex and *Theobaldia* are closely related organisms, in fact prior to 1902 *Theobaldia* was included in the genus *Culex*. The cytological differences between these two mosquitoes are thus more probably ascribable to quantitative rather than qualitative differences in the laws governing chiasma formation. The logical inference to be drawn from the comparison between them is that chiasma interference is fundamentally the same phenomenon whether it is working within one chromosome arm or across the centromere. *Theobaldia* has evidently a much lower chiasma interference than *Culex*, since its chiasma frequency is roughly twice as great. Thus even were chiasma formation of equal intrinsic likelihood at all points down the length of the chromosome, a more sensitive method of scoring would be required to reveal its action across the centromere. We have, however, good evidence that chiasma formation is not of equal intrinsic likelihood in the various parts of the chromosome (Mather, 1938). This author has designated as the 'differential distance' the mean distance from the centromere at which the most proximal chiasma is formed; and as 'interference distance' the mean distance between adjacent chiasmata in the remainder of the chromosome. Differential and interference distances are often visibly different in length. They seem to be determined by different factors; the older view that the centromere behaves as though it were itself a chiasma in determining the site of formation of other chiasmata appears to be incorrect. In the grasshopper *Mecostethus grossus*, for example, the distance of the proximal chiasma from the centromere seems to be limited quite precisely by the length of heterochromatin adjacent to the centromere. The chiasma falls just outside the heterochromatic region; since the amount of heterochromatin varies between the bivalents of the complement, the differential distance also varies, yet is more or less constant for each individual bivalent (White, 1945; Callan, unpublished).

Pätau has drawn attention to the way in which differential distance and chiasma interference can be expected to interact in determining chiasma distribution and correlation. The region comprised by the differential distance may be one in which chiasma formation is inhibited by some factor such as tardy division of the chromosomes. Symmetrical variation of this distance about the centromere could explain those cases where positive correlation of chiasmata or cross-overs on either side of the centromere has been observed. Proximal localization of pairing could produce the same effect. Moreover, unless chiasma interference is so potent that it can exert an effect not only across the centromere but also across the two differential distances contiguous with it, then clearly interference will be masked by the positive correlation determined by other factors. It is equally clear that we should not expect to find chiasma interference across the centromere in chromosomes where pachytene pairing is incomplete and does not include the centromere regions.

Pätau has also considered the problem of chiasma interference across the centromere in connexion with relational coiling of the chromosomes at pachytene. On Darlington's theory of chiasma formation (Darlington, 1935*b*) the mechanical instability of the chromosomes at the moment of their division or replication is conditioned by the relational coiling between partners. The formation of a chiasma releases the strain imposed by relational coiling. Chiasma interference is then understood as the distance between the

point where the strain was originally released and that where it again rises to a breakage threshold. On Darlington's theory, positive chiasma interference across the centromere can only arise if the direction of relational coiling is the same on either side of the centromere. If, on the other hand, the direction of coiling is opposite in the two arms, then the formation of a chiasma in one arm should increase the chance of formation of another in the other arm. The first-formed chiasma would act as a 'tie', thereby preventing that compensatory unravelling of the relational coiling about the centromere region which would otherwise occur.

Unfortunately, we know little about the direction of relational coiling at pachytene. In most organisms the chromosomes at this stage fix very poorly: moreover, the threads are near to the size limit of resolution and they are generally intertwined in a complex manner. Darlington (1935*a*, 1936*b*) has studied relational coiling at diplotene in *Fritillaria* and *Chorthippus*. He is led to infer that at pachytene there is a marked preponderance of bivalents with opposite directions of coiling in the two arms. These organisms have very large chromosomes in comparison with those of mosquitoes, and we would not be justified in the assumption that what holds for the one is true of the other also. Examination of pachytene in mosquitoes themselves cannot bring any evidence to bear on the question, since the nuclei are small and the chromosomes much intertwined. It is therefore not yet possible to reach any conclusions about the relationship between relational coiling at pachytene and chiasma interference across the centromere.

We have unpublished evidence of chiasma interference across the centromere in a number of other organisms. Thus the chromosomes of the dipteran *Psychoda* sp. almost invariably form a single chiasma in one arm only, though the centromeres are approximately median in position. In the earthworm *Eisenia foetida*, where five of the chromosomes have arms visibly unequal in length, chiasmata can be formed in long or short arms, yet it is most exceptional for them to be formed in both arms of the one bivalent.

The case of *Petunia violacea*, *P. axillaris*, and the hybrid between these two plant species is particularly instructive. Mather (1943*a*) has studied these plants from a genetical standpoint, and he kindly allowed one of us to examine meiosis in his material. Both *P. violacea* and *P. axillaris* have 14 chromosomes, superficially alike and with median centromeres. *P. violacea* has a mean chiasma frequency per bivalent of $1{\cdot}09 \pm 0{\cdot}02$. It is rare for more than one chiasma to form within an arm pair. The observed proportions of univalent pairs, one-chiasma and two-chiasmata bivalents deviate from the expected proportions (assuming arm equality and independence) in the same way as was found in *Culex*, i.e. bivalents with a chiasma in one arm only are disproportionately common. *Petunia axillaris* has a mean chiasma frequency per bivalent of $2{\cdot}06 \pm 0{\cdot}03$. The chromosomes of this species frequently form two chiasmata within the arm pair, and the proportions of the various types of bivalent agree closely with expectation in the same way as was found in *Theobaldia*. The hybrid between the two species has a mean chiasma frequency per bivalent of $1{\cdot}57 \pm 0{\cdot}02$, which is intermediate between the means of the parent forms. The rise in chiasma frequency relative to *Petunia violacea* is consequent on an increase in the number of bivalents which have formed one chiasma in both arm pairs, two chiasmata within a single arm pair being just as infrequent as in *P. violacea*. Since we cannot ascribe this greater regularity of chiasma formation to an increase in structural homozygosity—the opposite must be the case—a fall in the chiasma interference operating across the centromere appears to be the reasonable inference.

It is also possible to explain some observations of Frankel (1940) on various species of *Fritillaria* by assuming chiasma interference across the centromere. In the chromosomes with subterminal centromeres of seven out of eight species, Frankel found an inverse correlation between the number of chiasmata formed in the long arms and the presence or absence of a chiasma in the short arms. Since, however, the chromosomes of these species are very large and show, to a varying extent, incomplete pairing at pachytene, we should not overlook the possibility of this correlation being the result of a pairing phenomenon and not one of interference.

Finally, we must consider the genetic consequences of the chiasma interference which we find in *Culex*. In this organism, interference distance and chromosome length are critically balanced. Each chromosome forms one chiasma, or two when interference permits; the frequency with which two chiasmata are formed varies over a wide range in the specimens studied. Thus our ten specimens show a range of chiasma frequency per bivalent from 1·05 to 1·37, while Moffett found an even greater spread in his six specimens of *Culex*. The same relative variability in the occurrence of one more chiasma than normal will clearly lead to a wider overall range of variation when 'normal' is one chiasma than when it is two or more. We cannot define in precise terms what this exceptional variability in chiasma frequency will give rise to in terms of cross-over variability in specific regions of the chromosome. However, Gilchrist & Haldane (1947) have found an unusually high variability in cross-over frequency between eye colour and sex-determining genes in *C. molestus*, which is what we should also infer from our cytological observations.

6. The independence of the bivalents of *Culex pipiens* in chiasma formation

Culex pipiens is also a favourable object for the study of chiasma correlation between different bivalents within the same nucleus. Negative correlation between the chiasma or cross-over frequencies of different bivalents has been observed in a number of organisms, though it is by no means universal (Mather, 1936*a et al.*).

If the probabilities of formation of two chiasmata, one chiasma or no chiasma be assumed the same for '*M*' as for '*m*' chromosome pairs in a given individual, and if a, b and c are the numbers of two-chiasmata bivalents, one-chiasma bivalents and univalent pairs respectively, then in the absence of correlation the proportions of cells containing the various combinations of these types of bivalent are given by the terms of the trinomial expansion $(a+b+c)^3$. In these calculations, cells containing bivalents with two chiasmata in a single arm pair have been neglected. Table 11 shows the observed and expected numbers of the various cell types in specimens 2, 4, 9 and 10. These have been selected as examples, since in them the greatest numbers of cells were studied. Analyses of χ^2 show that in all ten specimens there is good agreement between observed and expected figures. (In the four specimens chosen as examples, $n=3$ in all cases and $P>0{\cdot}4$, 0·9, 0·8 and 0·9 respectively.) We may therefore conclude that there is no correlation between the bivalents of *C. pipiens* in the formation of chiasmata.

The χ^2 test does not take into account the sign of the deviation of observation from expectation, and thus any slight trend away from randomness cannot be made evident from this test alone. Table 12 shows the sign of the deviation from expectation for certain cell types in the ten individuals. Combinations involving univalents give unreliable figures, since the proportions of these cell types are always low in value. The sign of the

deviations of the remaining four combinations, which appears to be at random, shows clearly that: (1) there is no competition between bivalents within the same nucleus, and (2) high and low chiasma frequencies are not functions of the nuclei as units, i.e. the 'time-limit' to pairing (Darlington, 1936*a*, 1940), plays no part in the determination of the number of chiasmata in the nucleus of *C. pipiens*.

Table 11. *Culex pipiens*

(Figures of expectation are given in brackets.)

Cell type	Specimen no. 2	4	9	10
X/X X/X X/X	4 (2·41)	4 (3·10)	1 (0·54)	0 (0·68)
X/X X/X X/O	10 (14·48)	24 (24·64)	5 (7·28)	9 (8·99)
X/X X/X O/O	3 (0·63)	0 (0·00)	0 (0·13)	0 (0·13)
X/X X/O X/O	28 (28·98)	64 (65·51)	34 (32·34)	41 (39·49)
X/X X/O O/O	3 (2·52)	0 (0·00)	3 (1·17)	2 (1·18)
X/O X/O X/O	22 (19·32)	59 (57·85)	48 (47·87)	57 (57·86)
X/X O/O O/O	0 (0·06)	0 (0·00)	0 (0·01)	0 (0·01)
X/O X/O O/O	1 (2·52)	0 (0·00)	1 (2·59)	2 (2·58)
X/O O/O O/O	0 (0·10)	0 (0·00)	0 (0·05)	0 (0·03)
O/O O/O O/O	0 (0·00)	0 (0·00)	0 (0·00)	0 (0·00)
Totals	71	151	92	111

Table 12. *Culex pipiens*

Sign of deviation of observed from expected frequencies

Cell type	Specimen no. 1	2	3	4	5	6	7	8	9	10	Total +	Total −
X/X X/X X/X	−	+	+	+	+	−	+	−	+	−	6	4
X/X X/X X/O	−	−	+	−	+	−	−	−	−	−	2	8
X/X X/O X/O	+	−	−	−	−	+	+	+	+	+	6	4
X/O X/O X/O	−	+	+	+	+	+	−	−	+	−	6	4

7. SUMMARY

1. Analysis of chiasma distribution in the bivalents of the mosquito *Culex pipiens* shows that chiasma interference acts across the centromere in this organism. The strength of the interference varies from specimen to specimen.

2. This interference diminishes as the distance from the chiasma increases.

3. In the mosquito *Theobaldia* (*Allotheobaldia*) *longiareolata* chiasma interference is much weaker than it is in *Culex*. Its action cannot be detected across the centromere.

4. Comparison of the two mosquitoes leads to the inference that chiasma interference which crosses the centromere is the same phenomenon as chiasma or cross-over interference which is generally held to be confined within one arm on one side of the centromere.

5. There is no correlation between the chiasmata formed by different bivalents within the same cell of *C. pipiens*.

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REFERENCES

BARBER, H. N. (1941). Chromosome behaviour in *Uvularia*. *J. Genet.* **42**, 223.

BENNETT, E. S. (1938). The origin and behaviour of chiasmata. XIV. *Fritillaria chitralensis*. *Cytologia, Tokyo*, **8**, 443.

CALLAN, H. G. (1940). The chromosomes of the Cymothoid Isopod *Anilocra*. *Quart. J. Micr. Sci.* **82**, 327.

DARLINGTON, C. D. (1935*a*). The internal mechanics of the chromosomes. III. Relational coiling and crossing-over in *Fritillaria*. *Proc. Roy. Soc.* B, **118**, 74.

DARLINGTON, C. D. (1935*b*). The time, place and action of crossing-over. *J. Genet.* **31**, 185.

DARLINGTON, C. D. (1936*a*). The external mechanics of the chromosomes. *Proc. Roy. Soc.* B, **121**, 264.

DARLINGTON, C. D. (1936*b*). Crossing-over and its mechanical relationships in *Chorthippus* and *Stauroderus*. *J. Genet.* **33**, 465.

DARLINGTON, C. D. (1940). The prime variables of meiosis. *Biol. Rev.* **15**, 307.

FRANKEL, O. H. (1940). The causal sequence of meiosis. I. Chiasma formation and the order of pairing in *Fritillaria*. *J. Genet.* **41**, 9.

GAIRDNER, A. E. & DARLINGTON, C. D. (1931). Ring-formation in diploid and polyploid *Campanula persicifolia*. *Genetica*, **13**, 113.

GILCHRIST, B. M. & HALDANE, J. B. S. (1947). Sex linkage and sex determination in a mosquito, *Culex molestus*. *Hereditas, Lund*, **33**, 175.

GOWEN, J. W. (1919). A biometrical study of crossing-over. *Genetics*, **4**, 205.

GRAUBARD, M. (1932). Inversion in *Drosophila melanogaster*. *Genetics*, **17**, 81.

HALDANE, J. B. S. (1931). The cytological basis of genetical interference. *Cytologia, Tokyo*, **3**, 54.

KIKKAWA, H. (1935). Biological significance of coincidence in crossing-over. *Jap. J. Genet.* **11**, 51.

LA COUR, L. (1937). Improvements in plant cytological technique. *Bot. Rev.* **5**, 241.

LINDEGREN, C. C. & LINDEGREN, G. (1937). Non-random crossing-over in *Neurospora*. *J. Hered.* **28**, 105.

MATHER, K. (1936*a*). Competition between bivalents during chiasma formation. *Proc. Roy. Soc.* B, **120**, 208.

MATHER, K. (1936*b*). The determination of position in crossing-over. I. *Drosophila melanogaster*. *J. Genet.* **33**, 207.

MATHER, K. (1938). Crossing-over. *Biol. Rev.* **13**, 252.

MATHER, K. (1943*a*). Specific differences in *Petunia*. I. Incompatibility. *J. Genet.* **45**, 215.

MATHER, K. (1943*b*). *Statistical Analysis in Biology*. London: Methuen.

MOFFETT, A. A. (1936). The origin and behaviour of chiasmata. XIII. Diploid and tetraploid *Culex pipiens*. *Cytologia, Tokyo*, **7**, 184.

MULLER, H. J. (1916). The mechanism of crossing-over. II. *Amer. Nat.* **50**, 284.

NEWCOMBE, H. B. (1941). Chiasma interference in *Trillium erectum*. *Genetics*, **26**, 128.

PÄTAU, K. (1941). Cytologischer Nachweis einer positiven Interferenz über das Centromer (Der Paarungskoeffizient. I). *Chromosoma*, **2**, 36.

SCHWEITZER, M. D. (1935). Analytical study of crossing-over in *Drosophila melanogaster*. *Genetics*, **20**, 497.

STEVENS, N. M. (1910). The chromosomes in the germ cells of *Culex*. *J. Exp. Zool.* **8**, 207.

STEVENS, W. L. (1936). The analysis of interference. *J. Genet.* **32**, 51.

WHITE, M. J. D. (1945). *Animal Cytology and Evolution*. Camb. Univ. Press.

STRUCTURAL CHANGES PRODUCED IN MICROSPORES OF *TRADESCANTIA* BY α-RADIATION

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(With Two Text-figures)

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Introduction

The experiments about to be described constitute an extension of the series of investigations by Catcheside, Lea, Thoday and Kotval, of the chromosome structural changes produced in microspores of *Tradescantia bracteata* (clones 20^2 and 21^3) by X-rays and neutrons, to a study of the structural changes produced by α-particles.

The very extensive literature concerned with the structural changes produced by ionizing radiations has been reviewed from time to time, notably by Sax (1941), Giles (1943), Darlington & La Cour (1945) and Catcheside, Lea & Thoday (1946*a*, *b*).

Differences in methods of scoring, and sometimes of interpretation, render difficult a detailed quantitative intercomparison of the results of different schools. Since the differences between the effects of one radiation and another are essentially quantitative rather than qualitative, we have adopted identically the method of scoring of the Cambridge school, and have followed closely the elegant analytical methods of Lea and Catcheside in order to infer the primary breakage frequency characteristic of α-radiation.

Starting from Sax's (1940) original conception that all observed aberrations arise from chromosome or chromatid breaks primarily produced by the passage of an ionizing particle through the thread at the locus of the break, Lea & Catcheside (1942) have concluded that all the available data, including the effects of hard X-rays, soft X-rays, and neutrons, as well as a few observations on the effect of polonium α-rays on chromosomes undergoing pollen-tube mitosis, could be accounted for as follows. It was assumed that the production of a primary chromatid break in general requires that the ionizing particle should completely traverse the thread and in so doing should give rise to the production of a considerable number of ions. It appears that for a high probability of break production the number of ions formed within the thread must be about 20. The faster an electron is

moving the less dense the ionization along its track, and it is only when an electron has less energy than about 3 kV. that it will produce 20 ions in a chromatid $0{\cdot}1\,\mu$ in diameter. Thus, much of the energy of the electrons generated by hard X-rays is ineffective, and only the tails of the tracks have a high probability of break production. The recoil protons generated by neutrons are relatively slow-speed particles, and in the case of the protons generated by (Li-D) neutrons (Thoday, 1942) produce ions at an average rate of about 500 ions/μ over the whole track. These particles will leave about 50 ions within the chromatid thread and are estimated to have a break-producing efficiency ($P=0{\cdot}67$) approaching unity. The neutrons used by Giles in the Yale experiments were probably somewhat more energetic than those used by Thoday; those used in the Harvard experiments were considerably more energetic than either. It is therefore in keeping with the general theoretical interpretation offered by Lea and Catcheside that the faster Harvard neutrons were found (Giles, 1943) to produce fewer structural changes of all types per unit dose.

The α-particle is also a relatively slow-moving particle. In addition, it carries a double positive charge, and for this reason is even more densely ionizing than the recoil protons. The α-particles used in these experiments are estimated to produce on the average about 3500 ions/μ of track, that is, 350 ions in crossing a chromatid $0{\cdot}1\,\mu$ in diameter. This amount of ionization is so greatly in excess of the estimated number of ions required to produce a break that we may expect every α-particle transit through a chromatid to lead to a break. This, however, only makes the α-particle transit 50% more efficient than the (Li-D) recoil proton transit, and since, per unit of dose, the total length of track of the ionizing particles and therefore the number of intersections with the chromatid thread is inversely proportional to the ion density, a given dose of α-radiation should produce many fewer breaks than an equal dose of neutron radiation. Using the data already quoted,* the coefficient of primary break production for α-radiation should be at most $\frac{500}{3500}\times\frac{1}{0{\cdot}67}$ or one-fifth of that for (Li-D) neutrons.

This argument explicitly assumes that a particle passing near to but not through the thread is without effect. It is now known, however, that the inactivation of a number of biological entities may be brought about equally by ionization produced within the entity or by the ionization of the surrounding medium, though the number of ionizations required to bring about the inactivation process is generally very much greater in the latter case than in the former. This process of indirect inactivation though first demonstrated for simple inorganic molecules, has been shown to be effective in the inactivation by X-rays of enzymes (Dale, 1940, 1942, 1943; Lea, Smith, Holmes & Markham, 1944), and even of a body as large as the Shope papilloma virus, the diameter of which (hydrated) is three-quarters that of the estimated diameter of the chromatid thread in *Tradescantia*. If the chromatid threads can in fact be broken as a result of ionization produced in the surrounding medium, the effective target area presented by the thread is thereby increased, and in passing from neutrons to α-particles the number of aberrations produced per unit dose will not fall as abruptly as would otherwise be the case. In undertaking this investigation, therefore, we had in view not simply an extension of former investigations to particles of higher ion density, but the possibility of discovering whether at the highest

* When the δ-rays produced by the α-particles are taken into account, this figure is probably reduced by 20% as discussed on p. 147.

ion densities ionization of the surrounding medium makes any significant contribution to the total production of chromatid breaks. The evidence on this point is unfortunately not quite clear-cut, on the basis of the present observations. The number of visible chromatid aberrations produced by α-radiation, instead of being one-fifth of the number produced by an equal dose of neutrons, is actually somewhat greater; but owing to the difficulty of assessing the proportion of breaks which restitute, some doubt still exists as to the number of breaks primarily produced per unit dose of α-radiation, and therefore of the effective size of the target presented to an α-particle by the chromatid thread. It is, nevertheless, difficult to escape the conclusion that it is both greater than the target presented to a recoil proton, and corresponds to a thread diameter greater than the usual estimate of $0{\cdot}1\,\mu$. The experiments thus provide some evidence for a significant contribution to the total chromatid break production from ionization produced in the medium surrounding the thread when this ionization is produced by α-particles.

IRRADIATION TECHNIQUE

Physical technique

In principle the method of exposing microspores to known doses of α-radiation was the same as that used previously by Gray & Read (1942) for the exposure of root tips, and consists in immersing the specimen to be irradiated in a radon solution of known concentration. The radon, which belongs chemically to the group of inert gases, diffuses into the specimen until the concentration has reached an equilibrium value, which in the case of root-tip tissue is not measurably different from that of the surrounding fluid. The average concentration of radon within the specimen approaches equilibrium at a rate which is approximately exponential. In the case of a root tip 2 mm. in diameter, the time to reach half-equilibrium concentration was found to be $1\frac{1}{2}$ min. in agreement with an estimate based on the computed diffusion constant. For a specimen the size of the anthers used in these experiments, which were about 1 mm. in diameter, the concentration would reach half equilibrium value in about 0·4 min. When, at the end of the exposure, the inflorescences were transferred to clean water the radon would diffuse out at the same rate. The radon atoms which decay inside the anther become transformed into Ra A, which by emission of an α-particle having about the same range ($47\,\mu$) as the radon α-particle, changes into Ra B, which emits β-rays, and into Ra C′, which emits α-particles of $70\,\mu$ range. It was shown in the case of the root tips that these bodies, referred to collectively as the 'active deposit atoms', do not diffuse out when the specimen is transferred to clean water, but decay *in situ*, with time constants varying from 3 to 27 min. This, however, introduces no ambiguity into the estimate of dose provided the *radon* sensibly attains its equilibrium concentration throughout the specimen during the exposure. This was obviously true of all the exposure times used in the present experiment. The dose was calculated from the formula

$$\text{Dose in energy units} = 0{\cdot}73 \times \text{microcuries per c.c.} \times \text{exposure time in min.}$$

This unit of dose* is denoted by the symbol E, and the experimental data of Tables 1–3 are expressed in these units. Since Lea and Catcheside have already tabulated X-ray and neutron data in terms of 'röntgens', we have, for the purpose of intercomparison, expressed our results in Table 5 in aberrations per röntgen, assuming 1 r. = 1·15 E.

The average dose to which the microspores were exposed was about 20% higher than the figure given under the heading 'radon dose' for the following reason. The atoms of active deposit, which chemically resemble tellurium, bismuth and lead, tend to adhere to protoplasmic surfaces, and it was found in the root-tip experiments (Gray & Read, 1942) that after exposure to pure aqueous solutions of radon there

* The röntgen is strictly applicable only to X- and γ-radiations. To meet the need for a unit in terms of which neutron and other ionizing radiations could be measured, Gray, Mottram, Read & Spear (1940) introduced the energy unit of dose E, defined as follows. The unit of dose is that amount of radiation which produces an increment of energy in unit volume of tissue equal to the increment of energy produced in unit volume of water by 1 r. of γ-radiation. Unit dose corresponds to the production of roughly 2×10^{12} ions/c.c. of water or tissue.

was a considerable superficial accumulation of active deposit atoms which could be removed by gently wiping with cotton-wool. The extent of this accumulation was very greatly reduced when the radon was dissolved in 2% gelatin instead of pure water, and this procedure was used in the present experiments. The effect of the superficial layer of active deposit was allowed for by means of a control experiment in which inflorescences were immersed in 2% gelatin solution which had contained initially a fairly high concentration of radon, but from which the radon had been almost entirely removed by bubbling air through the solution just prior to the immersion of the inflorescences. One group of inflorescences was immersed for 20 min. and the other for 40 min., these being approximately the times used in the ordinary radon exposures. The concentration of Ra B and Ra C-active deposit atoms in the solution was measured throughout these control exposures by observing the strength of the emitted γ-radiations and the average value for each exposure was computed.

These concentrations of Ra B, Ra C and Ra C′ were many times as great as that of the active deposit present in solution during the principal radon exposures. In the first control experiment, for example, the concentration was equal to that which would be in equilibrium with a radon solution strong enough to deliver a dose of 68·5 E in 20 min., i.e. respectively 3·5 and 15·5 times as concentrated as in the solutions actually used. Owing to the fact that the radon was not quite completely removed from the solutions used in the active deposit control experiments, the inflorescences used in these experiments were also exposed to a small dose of radiation from radon. The amount of radon present was measured, and an appropriate correction applied. On account of its short life, practically all the Ra A originally present in the active deposit control experiments would have decayed before the introduction of the inflorescences. The observed aberrations were therefore produced by the Ra C′ α-particles alone. In the principal radon exposures there would no doubt be a small accumulation of Ra A on the surface of the anthers. Considering, however, that on the average only 3 min. elapses before the disintegration of Ra A, whereas an average of 50 min. elapses before the Ra C′ disintegration, it is evident that the atoms which disintegrate as Ra C′ on the surface of the anther will have been drawn from a much larger volume of solution than those which disintegrate as Ra A. The Ra C′ disintegrations will therefore be much more numerous than Ra A;* in addition, the Ra C′ α-particles penetrate considerably deeper into the anthers (cf. Fig. 1), and therefore affect more microspores. For these reasons it seems quite safe to assume that as regards the surface accumulation of active deposit the effects of the Ra C′ α-particles greatly preponderates, and hence that the active deposit control experiment does in fact simulate with sufficient accuracy the extent of the irradiation to which anthers are exposed in the principal radon exposures, due to the superficial layer of active deposit.

The concentrations of radon used in the main experiments are given at the head of Table 1. The amounts of dissolved radon were in all cases estimated by the γ-ray method during the actual exposures.

The anthers were exposed to a β-ray dose (from Ra B and Ra C) equal to about 5% of the α-ray dose. The aberrations produced by such a dose of β-radiation would be quite negligible compared with those due to the α-radiation.

Cytological technique

The treatments were carried out at the Mount Vernon Hospital, Northwood, Middlesex, on 30 September 1941.

Shoots, bearing inflorescences, of *T. bracteata* (clone 20²) grown at Cambridge, were cut the evening before and taken to Northwood, wrapped in moist towelling paper and enclosed in a tin.

While the radon solutions were being prepared the buds were stripped of their bracts, sepals and petals, to facilitate the access of the solution to the anthers, and to make the contact as direct and uniform as possible. The actual irradiations were done by immersing the inflorescences in the radon solutions for measured times. On removal from the solutions the inflorescences were carefully washed in pure water and then placed with their cut ends in water. The exposures were all carried out between 10.00 a.m. and 2.00 p.m., and the inflorescences were taken back to Cambridge the same day.

The fixations were made severally the next morning at definite time intervals, as nearly as possible 24 hr. after the irradiation. Smears of pollen grains at the first pollen-grain metaphase were fixed in

* Assuming each type of atom has the same mobility in gelatin solution and that all stick on reaching the anther, the number which decay as Ra A will be about 6% of those which decay as Ra C′.

Benda's solution. After fixation the slides were washed in water, graded to 70% alcohol, and then bleached with hydrogen peroxide in 80% alcohol. They were then stained by the crystal violet-iodine method and permanently mounted in Canada balsam.

The observations of metaphase plates were made mainly in polar view, but some obliquely placed plates were also recorded. In the latter case certain features of observations in chromosomes at lower foci were sometimes obscured, and in such cases the divisions were discarded from the records. At the higher doses where more aberrations per cell occurred the proportion discarded was somewhat higher. The discarded

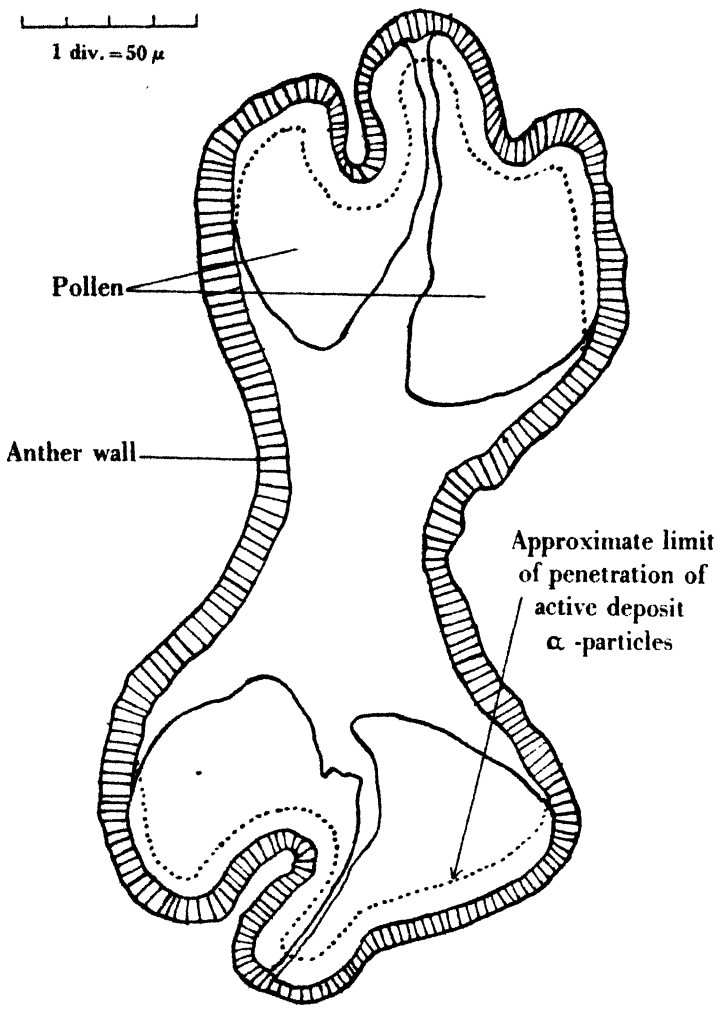

Fig. 1. Section of an anther showing location of pollen and approximate limits of penetration of α-particles from the superficial layer of active deposit.

divisions may well not have been a random sample of all the divisions within a slide, but have included a disproportionate number of those with several aberrations. These discarded divisions amount to about 0·2% of the total number of cells counted at lower doses, and to about 1·0% at higher doses.

The bias in discarding divisions, though relatively slight, may go some way towards accounting for the observed proportions of aberrations at high doses being somewhat lower than expected from the proportions at lower doses.

Analysis of the observed structural changes

The types of structural change observed and their relation to dose

The types of aberration seen at metaphase 24 hr. after exposure to α-radiation were identical with those seen at the same interval after exposure to X-rays (Sax, 1938, 1940; Catcheside, Lea & Thoday, 1946*a*) or neutrons (Thoday, 1942; Giles, 1941).

Reasons have been given by Catcheside and Lea for believing that the aberrations seen at metaphase 24 hr. after medium doses of X-rays (~150 r.) arise almost entirely from chromatid injuries produced in early prophase, when the chromosomes are effectively split. Splitting is estimated to have occurred about 6 hr. previously, that is, 30 hr. before metaphase, in irradiated material. The mitotic process in *Tradescantia* microspores is known to be prolonged by moderate doses of X-rays (Koller, 1943). Corresponding observations have not been made with α-rays, but in other material* it is known that α-rays have less effect on mitotic inhibition than an equal dose of X-rays. Moreover, in the present experiments the doses used were very small (4–40 *E*). It is possible, therefore, that at the time of irradiation the cells were at a somewhat earlier stage of prophase than in the corresponding X-ray and neutron experiments, and that a few aberrations may have arisen from the breakage of unsplit chromosomes.† The aberrations have, however, all been classified as if they were due to the action of radiation on split chromosomes. The experimental results are presented in Tables 1–3.

Table 1. *Results of examination of metaphase plates 24 hr. after inflorescences were immersed in radon solution*

							Interchanges					
							Complete involving chromosomes					
Dose *E*	Duration of exposure min.	No. of slides examined	No. of cells	Normal cells	Chromatid breaks	Isochromatid breaks	2	3	4	Incomplete	Total	Intra-changes
4·3	19	2	535	452	55	52	14	—	—	2	16	1
9·3	51·5	2	674	482	134	140	24	—	—	10	34	4
19·0	20·5	2	353	221	139	148	23	2	—	9	36	3
41·7	51	2	402	151	285	316	51	5	—	18	79	8
'Active deposit' control												
A.D. = 68 + R.D. = 2·5	20	2	463	371	98	107	15	1	—	4	21	5
A.D. = 115 + R.D. = 5·2	40	2	518	298	168	172	23	3	1	6	38	8

The symbols R.D. and A.D. refer to radon dose and active deposit dose respectively, and are to be understood as follows: In the first active deposit control experiment inflorescences were exposed to a radon dose (R.D.) of 2·5 *E* due to radon, and to active deposit (A.D.) equal in amount to that to which they would be exposed in the course of a radon dose of 68 *E*. In the second experiment the actual radon dose (R.D.) was 5·2 *E*, and the active deposit (A.D.) was equal in amount to that which would accompany a radon dose of 115 *E*.

It will be seen from Table 2 and Figs. 1–3 that, as judged by the χ^2 homogeneity test, the number of isochromatid breaks, and of interchanges, increases in direct proportion to the dose over the range tested. In the case of the chromatid breaks the observations do not fit a linear dose relation quite so well, rather too few aberrations being observed at

* *Vicia faba*; Gray, Mottram and Read (unpublished).

† In their 1940 series of experiments Catcheside, Lea & Thoday observed 18–23 hr. after irradiation 8 times the expected number of isochromatid-isochromatid interchanges. An *i/i* interchange is, however, indistinguishable at metaphase from an exchange between chromosomes unsplit at the time of irradiation. They point out that the discrepancy would be explained if it were assumed that 1% of the chromosomes were unsplit at the time of irradiation. The proportion may therefore have exceeded 1% in the present experiments.

the highest dose.* The deviation is probably not significant. Values of χ^2 as great as three times the number of degrees of freedom have not infrequently been found by other workers† and apparently arise from an interslide variation of unknown cause. In these experiments only two slides were used for each dose, and the value of χ^2 is estimated solely on the basis of the number of aberrations scored.

It has now become well established that chromatid breaks are produced by X-rays and neutrons in direct proportion to the dose, and the present experiments substantially confirm that the same is true for α-radiation.

Table 2. *Final estimates of aberrations per* 100 *nuclei*

		Dose				Aberrations per 100 cells per unit dose	Test for goodness of fit		
		4·3 *E*	9·3 *E*	19·0 *E*	41·7 *E*	*m*	χ^2	D.F.	*P*
Chromatid breaks	Observed	10·13	19·85	39·40	71·10				
	Active deposit correction	0·88	2·36	4·15	9·81				
	Breaks due to homogeneous α-radiation	9·25	17·49	35·25	61·29	1·70±0·08	10·2	3	0·02
Isochromatid breaks	Observed	9·73	20·75	41·90	78·50				
	Active deposit correction	0·88	2·07	4·41	10·31				
	Breaks due to homogeneous α-radiation	8·85	18·68	37·49	68·19	1·83±0·08	6·1	3	0·1
Total interchanges	Observed	2·99	5·03	10·22	19·70				
	Active deposit correction	0·19	0·28	0·55	1·82				
	Breaks due to homogeneous α-radiation	2·80	4·75	9·67	17·88	0·47±0·04	2·8	3	0·45

Table 3. *Subclassification of isochromatid breaks and interchanges*

	Isochromatid aberrations					Interchanges		
Dose	*SU*	*NUp*	*NUd*	*NUpd*	Total	Complete	Incomplete	Total
4·3	14	1	2	46	63	14	2	16
9·3	20	8	11	85	124	24	10	34
19·0	16	7	6	62	91	27	9	36
41·7	21	11	13	93	138	61	18	79
Total	71	27	32	286	416	126	39	165
%	17·1	6·5	7·7	68·7	—	75·5	24·5	—

Note. Of the 656 isochromatid breaks listed in Table 1, 286 were re-examined and classified as in Table 3. *SU* = sister union. *NUp* = non-union proximally. *NUd* = non-union distally. *NUpd* = non-union proximally and distally.

The fact that the number of isochromatid breaks is proportional to the α-ray dose is also in accordance with expectation, since this type of aberration is supposed, on the basis of a linear dose relation, to be produced by a single ionizing particle in the case of both X-rays and neutrons.

It is of special interest that the number of interchanges also varies linearly with the dose, indicating that in the majority of cases the two breaks which interchange are produced by the same ionizing particle. The two breaks which interchange are rarely produced by the same ionizing particle, in the case of X-rays, but are generally so produced in the case of material exposed to medium doses of neutrons (Thoday, 1942; Giles, 1943). *A fortiori*, in the case of low doses of α-radiation, in which, per unit of dose, there is a much smaller total length of ionizing track, two breaks which interchange would

* Cf. p. 139.
† Fabergé (1941) and Catcheside, Lea & Thoday (1946), Statistical note.

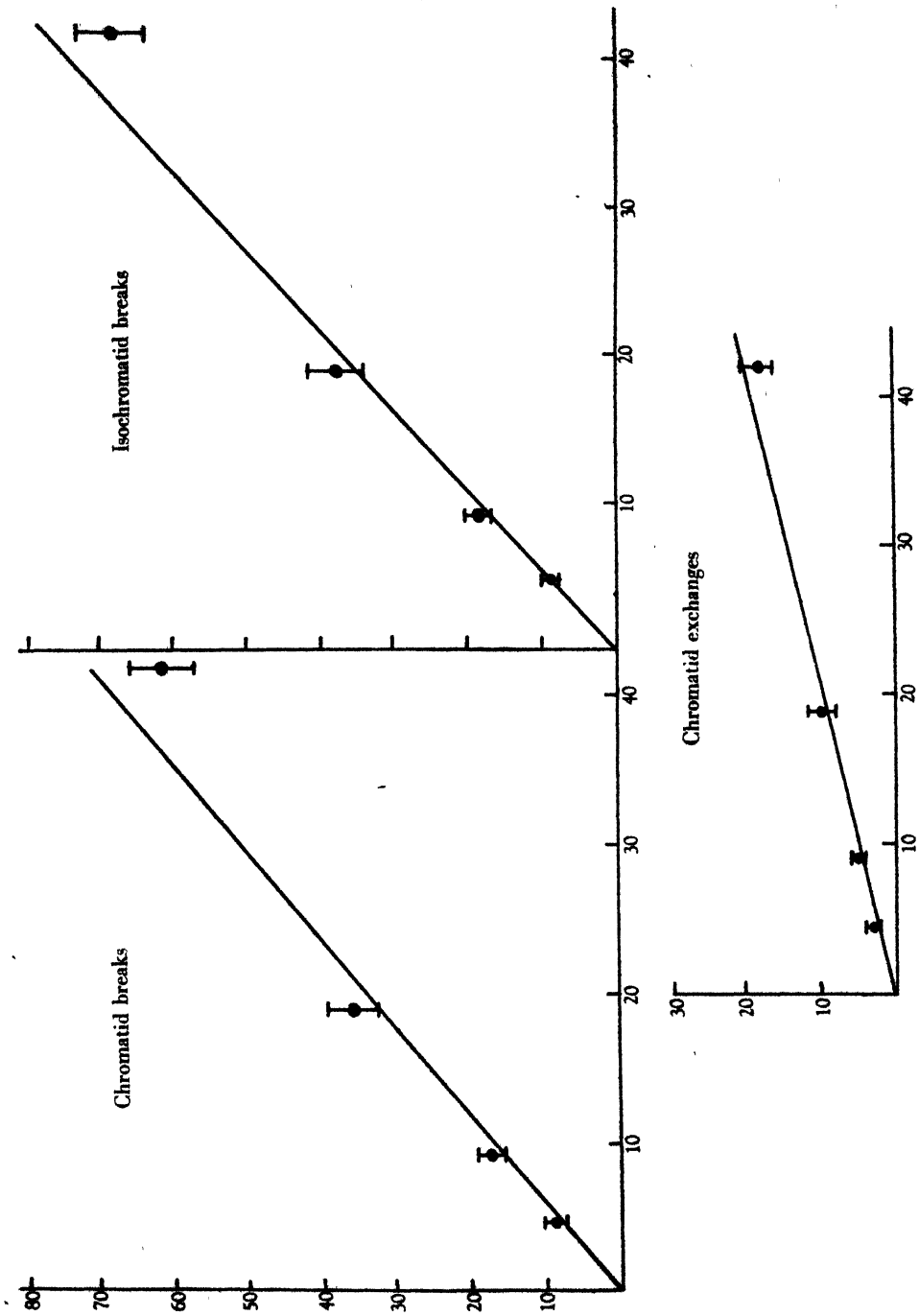

Fig. 2. Chromatid aberrations as a function of the α-particle dose. Abscissae: dose in energy units. Ordinates: aberrations per 100 cells.

be expected to be produced almost invariably by the same particle, leading to the observed linear variation of aberration frequency with dose.

In two respects, however, the results do not appear to fall into line with the observed effects of X-rays and neutrons; namely, in regard to the absolute number of each type of aberration produced per unit dose, and in regard to the extent to which broken ends fail to restitute. We will consider the latter point first.

The proportion of broken chromatid ends which do not undergo restitution or form new unions

Table 3 shows that a considerable proportion of the isochromatid aberrations show non-union of the proximal or distal fragment, or both, and that a quarter of the observed interchanges are incomplete. Thus the broken ends of many chromatid fragments produced at prophase have failed to reunite during the subsequent 24 hr. Following Catcheside, Lea & Thoday (1946*b*) we shall interpret this as indicating that when a chromatid is broken one or both ends may be so injured as to be incapable of taking part in a reunion, and we shall adopt their procedure for estimating P_1 and P_2, the respective probabilities of these events. The probability that an isolated chromatid break produced at prophase will restitute is $(1-P_1-P_2)$ and the probability that it will be seen at metaphase is (P_1+P_2), which Catcheside, Lea & Thoday denote by f. We may use one of their formulae to derive a value of f from the data given in Table 3, as follows:

$$f=\frac{NUp+NUd+NUpd}{2SU+NUp+NUd+NUpd}=\frac{27+32+286}{142+27+32+286}=0{\cdot}72.$$

The derivation of the above expression for f assumes implicitly that both sister chromatids are affected alike, i.e. both are either uninjured, injured proximally, or injured distally. Since the ratio of

$$\frac{(NUp+NUd)}{NUpd}=\frac{P_1}{P_2},$$

the isochromatid observations give

$$f=P_1+P_2=0{\cdot}72,\quad P_1=0{\cdot}12,\quad P_2=0{\cdot}60.$$

If it is assumed that there is no correlation between the type of injury sustained by sister chromatids, then a somewhat similar analysis leads to

$$f=P_1+P_2=0{\cdot}60,\quad P_1=0{\cdot}18,\quad P_2=0{\cdot}42.$$

Catcheside, Lea & Thoday (1946) point out that the experimental X-ray data confirm the existence of the following expected relationship between non-union isochromatid aberrations and incomplete asymmetrical interchanges:

$$\frac{NUp+NUd}{SU+NUp+NUd}=\frac{\text{Incomplete asymmetrical interchanges}}{\text{Total asymmetrical interchanges}}.$$

In the present investigation symmetrical and asymmetrical interchanges were not classified separately, but assuming that, as with X-rays, the proportion of incomplete interchanges is the same in both cases, the α-ray data give

$$\frac{NUp+NUd}{SU+NUp+NUd}=\frac{27+32}{71+27+32}=0{\cdot}46,\quad \frac{\text{Incomplete interchanges}}{\text{Total interchanges}}=\frac{39}{165}=0{\cdot}24.$$

The difference between these two figures is not likely to have arisen through sampling errors, and suggests that some at least of the observations are subject to systematic errors. The isochromatid aberrations are the more difficult to observe, and this subclassification is therefore the more suspect. There is, moreover, another reason for suspecting the isochromatid subclassification. It has been shown to yield a value of $f=0{\cdot}6$–$0{\cdot}7$. This implies that 60–70% of the chromatid breaks primarily produced are recorded, as compared with 9% in the case of X-rays and neutrons. That the disparity between the degree of restitution following break production by α-particles and neutrons is not as great as this is clear from the following consideration of the relative numbers of interchanges produced by the two radiations.

There is no reason to suppose that when four *uninjured* chromatid ends coexist within a given volume as a result of the production of two chromatid breaks, the chance of interchange formation will depend

on the nature of the radiation which produced the breaks. Therefore, modifying the formula developed by Lea and Catcheside* by the introduction of the factor $(1-f)$ to allow for the number of breaks having both ends uninjured, we arrive at the following relation between the coefficients of complete interchange production by neutrons and α-particles:

$$\frac{\theta_n}{\theta_\alpha}=\frac{\text{Complete interchanges produced by neutrons}}{\text{Complete interchanges produced by } \alpha \text{ particles}}=\frac{[(\xi-2c)^2\,(1-f)^2]_n}{[(\xi-2c)^2\,(1-f)^2]_\alpha}\frac{N_\alpha}{N_n},$$

when subscripts n and α refer to neutron and α-radiation, ξ = coefficient of chromatid break production, i.e. the number of chromatid breaks primarily produced per unit dose, c = the observed number of isochromatid aberrations per unit dose, $(\xi-2c)$ = number of chromatid breaks primarily produced which are not accounted for as observed isochromatid aberrations, and N = number of ionizing particles per unit area per unit dose.

Introducing experimental values as follows:†

$$\frac{\theta_n}{\theta_\alpha}=\frac{0{\cdot}0091\times 0{\cdot}84}{0{\cdot}0059\times 0{\cdot}75}=1{\cdot}72,$$

$$[(\xi-2c)^2\,(1-f)^2]_n=0{\cdot}19^2\times 0{\cdot}91^2=3{\cdot}0\times 10^{-2},$$

$$N_\alpha=0{\cdot}56\times 10^{-3}\ \alpha\text{-particles per } \mu^2,$$

$$N_n=4{\cdot}05\times 10^{-3}\ \text{protons per } \mu^2,$$

we find
$$[(\xi-2c)\,(1-f)]_\alpha=\sqrt{\left(\frac{3{\cdot}0\times 10^{-2}\times 0{\cdot}56\times 10^{-3}}{1{\cdot}72\times 4{\cdot}05\times 10^{-3}}\right)}=4{\cdot}9\times 10^{-2}.$$

But
$$[\xi-2c-2\theta]_\alpha=\left[\frac{\text{Observed chromatid breaks}}{f}\right]_\alpha=\frac{1{\cdot}96\times 10^{-2}}{f}.$$

Introducing the experimental values (Table 5) $\theta_\alpha=0{\cdot}59\times 10^{-2}$ and solving, we find $(\xi-2c)_\alpha=7{\cdot}3\times 10^{-2}$ and $f=0{\cdot}33$. If we allow for the fact that the effective value of N_α is about 20% greater than the figure given above on account of the associated δ-rays which are capable of breaking a chromatid, then the values become slightly modified to $(\xi-2c)=7{\cdot}7\times 10^{-2}$, $f=0{\cdot}30$.

We attach considerably greater weight to this estimate of f than to that based on the proportion of isochromatid rejoins, since the latter are often difficult to observe.

In the hope of clarifying this point, however, Dr Catcheside very kindly undertook to score the aberrations on two slides. We are indebted to him for undertaking this and for allowing us to include his observations in this paper (Table 4). In many cases his observations do not differ significantly from ours, and clearly show that the proportion of injured ends is much higher than among breaks produced by X-rays or neutrons. Catcheside's figures, however, show a significantly higher proportion of *SU* and lower proportion of *NUpd* than ours and lead to a lower value of f than we obtained by this method, namely $f=0{\cdot}41$.

From the proportion of *SU*, *NUp*, *NUd* and *NUpd*, we can subdivide f into P_1 and P_2, the respective probabilities that one or both ends of a given break are injured. It matters little whether we assume complete correlation or no correlation between the injuries to sister chromatids. The results are:

$$P_1=0{\cdot}28,\quad P_2=0{\cdot}13,\quad (P_1+P_2)=f=0{\cdot}41.$$

Table 4 includes data for *i/c* and *i/i* as well as *c/c* interchanges, and from the proportion of these three types of interchange an independent estimate of f may be obtained. A modified form of Catcheside, Lea & Thoday's (1946) treatment of the data has been used so that f may be derived from the proportions of *i/c* and complete *c/c* interchanges. We find in this way $f=0{\cdot}26\pm 0{\cdot}07$. The number of *i/i* interchanges is

* Lea & Catcheside (1942, p. 234):

$$\text{Number of 1-hit interchanges}=\frac{3\,(\xi-2c)^2\,Dh}{4\pi R^2 N},$$

where $h=\int_0^\infty H(X)\,dx$, D = dose, and R = radius of nucleus.

† All coefficients are expressed as aberrations per röntgen (cf. p. 141 and Table 5). Neutron data are those of Thoday (1942). The proportion of complete interchanges has been taken as 0·84 for neutrons and 0·75 for α-particles (Table 3).

too small to be used for the evaluation of f, but it is satisfactory to note that using the above value of f, the expected number of i/i interchanges is 2·6, in good agreement with the observed number 3 (Table 4, third column from right).

The proportion of incomplete interchanges observed by Catcheside is 0·38, and checks satisfactorily ($\chi^2=1{\cdot}7$, $n=1$, $P=0{\cdot}1$–$0{\cdot}2$) with the value of $\frac{NUp+NUd}{SU+NUp+NUd}$ which is 0·48. Since the proportion P_2 of chromatid breaks in which both ends are injured do not contribute any interchanges, we cannot derive an absolute value of f from the proportion of incomplete interchanges. We can, however, derive a minimum value, by assuming $P_2=0$. This value is $f=P_1=0{\cdot}25$. At the lowest estimate (Table 4), 19% of the isochromatid breaks are of the $NUpd$ type, so that P_2 cannot in fact be much less than the figure calculated from Catcheside's data (Table 4), namely, 0·13. It follows that f cannot be much less than 0·38.

In the light of the above discussion there appears little doubt but that our first estimate of f, as between 0·6 and 0·7, is incorrect. Four other methods have all yielded values

Table 4. *Structural changes produced by α-radiation*

Aberrations scored by D. G. Catcheside, for comparison with data given in Tables 2 and 3.

Dose E	No. of slides	Cells: Total	Cells: Normal	Chromatid breaks	Isochromatid breaks: Total	*SU*	*NUp*	*NUd*	*NUpd*
19	1	123	58	71	48	18	12	8	10
41·7	1	79	10	76	81	36	18	13	14
Total	2	202	68	147	129	54	30	21	24

Dose E	No. of slides	Chromatid-chromatid interchanges: Total	Symmetrical: Total	Symmetrical: Complete	Symmetrical: Incomplete	Asymmetrical: Total	Asymmetrical: Complete	Asymmetrical: Incomplete: Proximally	Asymmetrical: Incomplete: Distally	Iso-chromatid-chromatid interchanges	Iso-chromatid-isochromatid interchanges	Chromatid-chromatid intrachanges	Iso-chromatid-chromatid intrachanges
19	1	20	9	7	2	11	7	2	2	7	0	5	6
41·7	1	33	15	7	8	18	12	3	3	14	3	3	7
Total	2	53	24	14	10	29	19	5	5	21	3	8	13

between 0·26 and 0·41, each having a standard deviation of about 0·07. We shall adopt the values

$$f=P_1+P_2=0{\cdot}35\pm0{\cdot}06,\quad P_1=0{\cdot}24,\quad P_2=0{\cdot}11.$$

The values of P_1 and P_2 are, of course, less certain than that of their sum f.

If there is complete correlation between the injuries sustained by sister chromatids, then it follows by a simple calculation, using $f=0{\cdot}35$, that the proportion of primarily produced isochromatid breaks which are seen as isochromatid aberrations is $z=0{\cdot}67$. That there is at least a large measure of correlation between the injuries sustained by sister chromatids is evident from the following consideration. If there were no correlation at all then calculation shows that of the primarily produced isochromatid breaks 0·41 would be seen as isochromatid aberrations, and 0·38 would be recorded as simple breaks; that is, an isochromatid break would give rise to about equal numbers of observable aberrations of these two types. Since the numbers observed are in fact about equal, there would be none left to be accounted for by the ordinary process of simple break production, and we should have to conclude, on this hypothesis, that an α-particle almost invariably breaks both sister chromatids if it breaks either. This is inherently improbable. Moreover, of the simple breaks left over from isochromatid breaks only 55% would be capable of forming interchanges, and these interchanges would all be incomplete. It would not be possible to account in this way for the high proportion of observed complete interchanges. We conclude, therefore, that in the majority of cases there is correlation between the injuries.

The coefficients of aberration production

(*a*) *Chromatid breaks*

Each isochromatid break and each interchange represents two chromatid breaks, and the observed chromatid breaks represent a fraction f of those primarily produced. Therefore in the notation of p. 144 the coefficient of chromatid break production ξ is given by

$$\xi = 2c + 2\theta + \frac{\text{Observed breaks}}{f} = 0{\cdot}042 + 0{\cdot}012 + \frac{0{\cdot}0196}{0{\cdot}35} = 0{\cdot}110.$$

(*b*) *Isochromatid breaks*

If ϕ is the coefficient of primary isochromatid break production, i.e. the number of occasions per cell per röntgen on which a pair of sister chromatids are broken at the same locus,

$$\phi = \frac{\text{Observed isochromatid aberrations}}{z} = \frac{0{\cdot}021}{0{\cdot}67} = 0{\cdot}031.$$

The coefficient z, which is the ratio of the observed isochromatid aberrations to the isochromatid breaks previously produced has been taken as 0·67 for α-particles instead of the figure 0·53 estimated by Lea & Catcheside for X-rays and neutrons to allow for the greater proportion of breaks incapable of restitution which favours the detection of isochromatid breaks.

It is at once evident from a comparison of the neutron and α-particle data (Table 5), that the aberrations estimated to be primarily produced by α-particles are far more

Table 5. *Structural changes observed at metaphase of the pollen-grain division of* Tradescantia *24 hr. after irradiation by X-rays, neutrons and α-particles*

The figures indicate the number of aberrations per cell per rontgen, assuming 1 r. of hard X-rays = 0·97 E, 1 r. of neutrons = 1·02 E, 1 r. of α-rays = 1·15 E.

Data derived entirely from inflorescences irradiated at Cambridge or Northwood.

X-rays and neutrons: Experiments by Catcheside and Lea, Thoday, and Kotval, see Catcheside, Lea & Thoday (1946).

α-particles: present experiments.

		Hard X-rays $f=0{\cdot}09$, $z=0{\cdot}53$	(Li-D) neutrons $f=0{\cdot}09$, $z=0{\cdot}53$	α-particles $f=0{\cdot}35$, $z=0{\cdot}67$
Chromatid breaks	Observed at metaphase	$0{\cdot}0072_5 \pm 0{\cdot}0008$	$0{\cdot}0158 \pm 0{\cdot}0008$	$0{\cdot}0196 \pm 0{\cdot}0009$
	Estimated to be produced at prophase, ξ	0·09	0·21	0·11
Isochromatid breaks	Observed at metaphase	$0{\cdot}0027_1 \pm 0{\cdot}0002$	$0{\cdot}0099 \pm 0{\cdot}0003$	$0{\cdot}0210 \pm 0{\cdot}0009$
	Estimated to be produced at prophase, ϕ	0·0051	0·019	0·031
Chromatid exchanges	Observed at metaphase, θ		$0{\cdot}009 \pm 0{\cdot}0003$	$0{\cdot}006 \pm 0{\cdot}0005$

numerous than would be expected in view of the fact that the total length of α-particle track per röntgen is only about one-seventh of that of the recoil protons produced by (Li-D) neutrons. The influence of the δ-rays produced by the α-particles has to be considered. Lea (1946) has estimated that the total length of δ-ray track associated with 1 μ of α-particle track is about 1·6 μ. A great deal of this total length of δ-ray track is, however, ineffective from the point of view of chromatid break production, for while the individual δ-rays have all energies up to 3·5 kV., there is a large preponderance of particles which have too short a range to cross the chromatid thread, and which, therefore, have a low efficiency of break production. The proportion of the total length of δ-ray track which is effective has been calculated using the criteria of Lea & Catcheside (1942) for break

production, and it is concluded that the δ-rays associated with the α-rays used in these experiments may be allowed for by taking the total track length to be 20% greater than the sum of the α-particle ranges. The total length of α-particle track per cubic micron* per röntgen is therefore taken to be $1{\cdot}2 \times 5{\cdot}6 \times 10^{-4} = 6{\cdot}7 \times 10^{-4}\,\mu$.

Derivation of the size of the effective target presented by the chromatid thread to an α-particle

The effective diameter of the target presented by a chromatid thread to an α-particle may now be estimated as follows. The total length of the 12 chromatids at prophase is estimated to be $972\,\mu$, so that if the effective diameter of the thread for chromatid break production is $2r$, the total length of α-particle track within the chromatids, when the cell is exposed to 1 r. of radiation is $972 . \pi r^2 . 6{\cdot}7 \times 10^{-4}\,\mu$. The average length of each transit is $2r$. Hence, equating the number of transits to the number of breaks produced by 1 r. of α-radiation (Table 5)

$$\frac{972 . \pi r^2 . 6{\cdot}7 \times 10^{-4}}{2r} = 0{\cdot}11 \quad \text{or} \quad r = 0{\cdot}11\,\mu;$$

$$\text{Effective diameter } 2r = 0{\cdot}22\,\mu.$$

This estimate is much greater than the usually accepted value of $0{\cdot}1\,\mu$.

That the target presented to α-particles for the production of a chromatid break is effectively greater than that presented to recoil protons is evident from a comparison of the α-particle and neutron coefficients of break production. By reasoning not involving an assumed diameter of the chromatid thread, Catcheside and Lea arrive at the conclusion that the thread is broken on 60% of the occasions on which it is traversed by a recoil proton generated by (Li-D) neutrons. Lea estimates that these neutrons generate a total length of recoil proton track, per cubic micron of tissue, of $4{\cdot}05 \times 10^{-3}\,\mu$/r., or $\frac{4{\cdot}05 \times 10^{-3}}{6{\cdot}7 \times 10^{-4}} = 6$ times the length of α-particle track per micron. The ratio of the coefficient of primary chromatid break production by α-particles and neutrons should not exceed $\frac{1}{6} \times \frac{1}{0{\cdot}6} = 0{\cdot}28$ if the thread presents the same target to each kind of particle. Similarly, for isochromatid break production the calculated upper limit is $\frac{1}{6} \times \left(\frac{1}{0{\cdot}6}\right)^2 = 0{\cdot}47$. These ratios may be compared with the values derived from the experimental data (Table 6).

Table 6

	Ratio of coefficients of break production by α-particles and neutrons		
	Calculated upper limit	Experimental	Experimental / Calculated
Chromatid breaks	0·28	0·52	1·9
Isochromatid breaks	0·47	1·63	3·5

A consideration of the relative efficiencies of the two radiations in primary break production thus leads to the conclusion that the target presented by the chromatid thread

* The average specific gravity of the cell is assumed to be unity.

to α-radiations has an apparent diameter at least twice as great as that presented to a recoil proton.

It is noteworthy that compared with protons, α-particles are relatively more efficient in isochromatid than in chromatid break production, since this result is to be expected if the effective size of the target is greater than the actual size of the thread. Indeed, a cogent argument in favour of the view that the chromatid thread may be broken by an α-particle which passes near but not actually through the thread may be derived from the α-particle results alone, as follows.

If the two chromatid threads be imagined as two cylinders lying parallel and close together, and an ionizing particle has a probability p of breaking a thread which it traverses, the ratio of the number of primary isochromatid breaks to the number of primary chromatid breaks is $\frac{1}{2}pg$* where g is the probability that a straight line drawn at random through one cylinder also passes through the other. The value of g does not depend on the size of the cylinders but only on the ratio of the radius of each to the distance between the axes. It is maximum and equal to 0·36 when the cylinders are in contact. Thus if actual transits are necessary for the production of breaks

$$\frac{\text{Coefficient of isochromatid production}}{\text{Coefficient of chromatid production}} = \frac{\phi}{\xi} < \tfrac{1}{2}pg < 0{\cdot}18.$$

This inequality is satisfied in the case of neutrons for which $\frac{\phi}{\xi} = \frac{0{\cdot}019}{0{\cdot}21} = 0{\cdot}09$ but not in the case of α-particles for which $\frac{\phi}{\xi} = \frac{0{\cdot}031}{0{\cdot}011} = 0{\cdot}28$.

Thus, regarded from three different points of view, the assumption that an α-particle must pass through the chromatid thread in order to break it, leads to numerical inconsistencies, amounting to factors of between 1·6 and 3·5. These inconsistencies are so large that they can hardly be attributed to errors in the estimated coefficients of production of the three main types of aberration by α-particles. Nor can they be removed by a change in the estimated value of the injury coefficient f, since a change in f which would remove one discrepancy would accentuate another. We incline therefore to the view that a proportion of the chromatid breaks produced by α-particles arise from ionization produced in the immediate vicinity of, but not within, the thread. It appears that α-particles passing at a distance from the axis equal to twice the radius of the thread have a high probability of breaking the thread.

The occurrence in nature of structural changes produced by α-radiation

Radioactive elements are very widely dispersed in minute amounts throughout the whole of nature. They are derived from the parent elements uranium and thorium, and to a lesser extent from protactinium, which are present in measurable amounts in all types of rock. The γ-radiation from this radioactive material present in the earth's crust produces about 3 ions/c.c./sec. in air over land areas. The γ-ray intensity over the sea is negligible by comparison, since the radioactive content of sea water is in general about a thousand times smaller than that of an equal mass of the earth's crust. The radio elements from the rocks very slowly pass into solution in the soil moisture and then into plants. The gaseous radio elements, radon and thoron, diffuse into the soil air, and are carried

* Cf. Lea & Catcheside (1942).

into the lower atmosphere. Thoron, having a half-life of less than a minute, remains very localized. But radon, which has a half-life of 3½ days, is carried by winds for immense distances over land and sea. The ionization arising from the radon and its decay products in the atmosphere at sea-level averages nearly double that due to the radiation from the earth's crust, and is rather more than the combined effect of γ-radiation and cosmic radiation; moreover, the ionization is mainly produced by α-radiation. The radon content of stagnant underground air or soil air is of course very much higher. For example, Sanderson (1911) found that 1 c.c. of air drawn from the depth of the soil in the neighbourhood of New Haven, U.S.A., contained $2{\cdot}4 \times 10^{-13}$ curies of radon. Concentrations ranging from $0{\cdot}01$–$5{\cdot}7 \times 10^{-13}$ curies/c.c. have recently been observed at a variety of localities in Holland by Sizoo, Sanders, Friele & van der Maas (1941). These authors also observed the presence of thoron in many of the samples of air examined but did not estimate it quantitatively. It is likely that thoron and its decay products contribute about as much ionization as radon and its products, but on account of its very short life, and therefore restricted diffusion range, thoron is probably less important biologically. A concentration of 2×10^{-13} curie/c.c. is about 2000 times greater than the average for atmospheric air. Since the radon in the soil air is derived from that in the soil moisture we may readily estimate the order of magnitude of the concentration of radon in the water surrounding the roots of plants, and hence the concentration in the roots, and the consequent dose rate of α-radiation to which the root will be exposed. The dose rate works out at about 10^{-4} energy units per day,* or of the same order as the γ-ray dose rate at the surface of the earth. The rapid transport of water through plants probably results in the aerial parts of small plants being exposed to an α-ray dose rate which is an appreciable fraction of that in the roots, and greatly in excess of that due to the radon diffusing into the plant from the surrounding atmosphere. Burkser, Brun & Bronstein (1927) found that a radioactive gas, almost certainly radon, was given off in small amounts by fresh leaves of meadow grass, *Iris* and other plants, but not from wilted or dried leaves.

Radium and thorium elements have been observed to be widely distributed throughout the plant and animal kingdoms. Unlike radon they tend to be selectively absorbed in particular organs. Radium, for example, which is homologous with calcium, concentrates ultimately in the skeletons of vertebrates. Pelz (1939) observed relatively high concentrations of radium in a number of woods, and found the radium to be situated mainly in the cortex and in the inner bark adjacent to the wood. The average radium or thorium content of a tissue may therefore give a very erroneous idea of the dose of α-radiation to which particular cells of the tissue are exposed. The figures given in the last column of Table 7 may nevertheless be of some interest as giving a rough guide to the average dose rates obtaining in various tissues; with regard to the dose due to radium, a crude approximation to that obtaining in particular types of cell could probably be obtained from these figures by assuming proportionality between the radium and the calcium concentration throughout a given tissue. Kunasheva (1939) showed that in the case of the pea the concentration of radium in the plant is proportional to that in the nutrient medium, over the range 10^{-11}–10^{-8} g. Ra/g. of solution. Thorium, and radiothorium which is isotopic with thorium, and from which the daughter elements of the thorium series grow with a half-life of about

* The surface cells of a root not protected by a film of moisture $\sim 50\,\mu$ thick, or the whole bulk of a micro-organism a few microns in size, situated in an air cavity in the soil not less than a centimetre in diameter, would be exposed to several hundred times this dose rate of α-radiation.

3½ days, have no chemical homologue among the more abundant constituents of living matter. We therefore have little guide as to their probable distribution. This is particularly unfortunate, since the figures given in the table show that as regards the average ionization throughout the tissues investigated that due to the disintegration products of thorium is in general about 10 times that due to radium and its products.

Potassium and rubidium are also radioactive, emitting β-radiations. The concentration of rubidium is so small in the tissues which have been investigated that its radioactivity may be neglected. The β-ray dose rate in a tissue containing 0·5 % by weight of potassium is about 5×10^{-5} E/day. The effects of the potassium content of tissue are likely therefore in general to be small compared with those of the γ-radiation from the earth's crust, and cosmic radiation.§

Table 7

Material	Radium or radon content of 1 g. of material in g. of Ra element or curies of radon*		Thorium content of 1 g. of material in g. Th element		Dose rate in energy units per day
	Range	Mean	Range	Mean	
Rocks (1) (igneous and sedimentary)	$0{\cdot}1–5 \times 10^{-12}$	$1{\cdot}5 \times 10^{-12}$	$0{\cdot}4–2{\cdot}5 \times 10^{-5}$	$1{\cdot}5 \times 10^{-5}$	—
Soil moisture (2) (radon content)	$0{\cdot}01–2 \times 10^{-13}$	10^{-13}	—	—	10^{-4}
Ocean sea water (3)	$0{\cdot}1–3 \times 10^{-16}$	10^{-16}	—	—	—
Marine plants and animals (3)	—	10^{-14}	—	—	10^{-5}†
Terrigenous mud, ocean-bottom deposit (4)	—	$2{\cdot}5 \times 10^{-12}$	—	—	—
Plants, fruits, vegetables, ox-flesh and milk (5)	$1{\cdot}5–50 \times 10^{-15}$	5×10^{-15}	$1–20 \times 10^{-7}$	5×10^{-7}	(radium) 5×10^{-6} (thorium) 10^{-4}
Skeleton of man (6)	—	10^{-12}	—	—	10^{-8}‡
Human tissues (7)	$10^{-12}–10^{-13}$	5×10^{-13}	—	—	—
Hence average for whole body	—	5×10^{-13}	—	—	—

* Unless otherwise stated, the figures refer to the content of radium element.
† Assuming the organism to be more than 50 μ in diameter.
‡ Actively proliferating cells within bone may be exposed to a very much smaller dose rate.

(1) Rutherford (1913).
(2) Sanderson (1911) and Sizoo, Sanders, Friele & van der Maas (1941).
(3) Evans, Kip & Moberg (1938).
(4) Evans & Kip (1938).
(5) Burkser, Brun & Bronstein (1927).
(6) Janitzky, Krebs & Rajewsky (1938).
(7) Krebs (1939).

Most land organisms will thus be exposed to two types of ionizing radiation, namely, β-radiation of fairly constant amount generated by cosmic rays and γ-rays (and potassium), totalling about 2×10^{-4} r./day (or 2×10^{-4} E/day), and α-radiation from radium, thorium and their disintegration products. The α-ray dose rate to which different cells are exposed is likely to vary greatly with the situation of the cell. It may be much smaller than the β-ray dose rate. In root tips it is likely to be of the same order as the β-ray dose, and in some tissues it may be very much larger. Other things being equal it will be higher in tissues such as seeds which have a high mineral content per unit mass. In very special circumstances, such as micro-organisms in large soil cavities, it may be several hundred

§ *Note added in proof.* Since the decay period of potassium is estimated (Thompson & Rowlands, 1943) to be about 400 million years, it is possible that the relative abundance of the radioactive isotope of potassium (K^{40}) was considerably higher during the early stages of evolution, and that the dose rate due to the β-rays of potassium exceeded the combined effect of γ-rays and cosmic rays.

times greater than 2×10^{-4} E/day. In assessing the relative importance of these two types of radiation in producing chromosome structural changes under conditions prevailing in nature, we have to remember that at low dose levels and at low dose rates about 6 times as many structural changes are produced by α-radiation as by an equal dose of β-radiation. If only two-hit aberrations, such as inversions and interchanges, are considered, α-radiation is probably more nearly 30 times as efficient as β-radiation.* Thus, the α-radiation is relatively most efficient in producing those structural changes which are the ones most likely to be of significance from the standpoint of the evolution of species.

Chromosome structural changes very closely resembling those produced by ionizing radiations have frequently been observed in plants and animals of many genera which have not been deliberately irradiated. The possibility that these might arise through the action of the γ-radiations emitted by soil and rocks was considered by Giles (1940). In order to compare the spontaneous aberration frequency with that induced by γ-radiation he exposed inflorescences from two plants of *Tradescantia* to the γ-rays from radium for 24 hr. at dose rates 10 times and 1000 times that of the local natural γ-radiation. At the higher dose rate, the total dose delivered was stated to be 2·2 r., which was expected to produce 0·05 % aberrations per chromosome in microspores examined at mitotic metaphase. No structural changes were seen in the plant in which no spontaneous aberrations had previously been observed, and in the other plant the number of aberrations scored (3/1344 or 0·22 %) in the irradiated inflorescences was not significantly different from those in controls (4/1578 or 0·26 %). The spontaneous aberrations could not therefore be ascribed to local γ-radiation. Other observations made at the same time, as well as later experiments (Giles, 1941), pointed to a powerful genetic control of the frequency of the spontaneous aberrations, since microspores from hybrid plants, and particularly a triploid hybrid, formed by a cross between a diploid and a tetraploid plant, showed a much greater aberration frequency than either parent.

When root tips from the same plants were examined, however, the aberration frequency was very much lower and no appreciable difference was found between the hybrid and either parent. In all, 58,000 chromosomes were examined. The primary aberrations observed consisted of two chromatid dicentrics, each accompanied by an acentric fragment, and one pair of acentric fragments indicative of a chromosome terminal deletion. Eight chromatid dicentrics unaccompanied by fragments were ascribed to the fusion of broken ends of previous bridges. Thus the total primary aberration frequency was roughly 0·005 %, and the primary chromatid aberration frequency about 0·003 %. It is of interest to compare this with the frequency which might be expected due to radon in the soil. The rate of production of chromatid dicentrics by α-particles in microspores has been found to be 0·7 % per energy unit. The α-ray dose rate due to radon and thoron in the soil has been estimated at about 2×10^{-4} E/day, so that if the efficiency of isochromatid break production were the same in roots as in microspores, the time necessary for the accumulation of a dose which would give rise to the observed spontaneous aberrations would be about 36 days. The actual time available (per mitotic cycle) is unlikely to exceed a tenth of this figure, but considering the statistical errors in the cytological observations and the crudeness of the radon dose estimate, the unknown influence of temperature and

* Catcheside, Lea & Thoday (1946*b*) estimate the number of chromatid exchanges per cell per röntgen produced by X-rays at low doses and low dose rates to be 2×10^{-4}. The number produced by α-rays (Table 5) is 6×10^{-3}. No corresponding data are available for aberrations resulting from the irradiation of resting nuclei.

other factors, it is evident that α-radiation from naturally occurring radio elements in the soil may well give rise in roots to an aberration frequency approaching that actually observed. Nichols (1941) observed a chromatid aberration frequency of 0·13 % in the root tips of a 5 months old *Allium* plant. About a third of the chromatid aberrations consisted of dicentrics with fragments, i.e. isochromatid breaks. The observed spontaneous frequency of this particular type of aberration is thus about 8 times that found by Giles in *Tradescantia* root tips.

Certain circumstances connected with the accumulation of aberrations in dormant seeds are suggestive of the action of ionizing radiations. Nichols (1941) studied the aberrations seen in the first root-tip mitosis of germinating seeds of several varieties of *Allium*. The aberrations were indistinguishable from the one-hit and two-hit chromosome and chromatid aberrations produced by X-rays. In almost every case the proportions of chromosomes showing aberrations increased with the period of storage, and in the case of the two varieties most fully investigated (Yellow Strassburg and Sweet Spanish) this increase was rather accurately proportional to the storage time over a period of 5 years, as would be the case in a cell of constant sensitivity exposed to a radiation of constant dose rate.

In Yellow Strassburg the observed aberration corresponded to a rate of production of $2{\cdot}7 \times 10^{-6}$ aberrations per chromosome per day of storage, which was about the average for the eight varieties studied. Although the proportions of chromosomes exhibiting aberrations is 6 times greater in the first root-tip mitosis after germination than in mitoses of adult root tips, the implied rate of aberrations per day is far smaller in the dormant seed than in adult tissue. Fewer root-tip aberrations, in the ratio of about 1 : 3, are produced by a given dose of X-rays by raying *Allium* seeds than by raying mature roots (Sax, 1941). On the other hand, the concentration of radioactive material would be expected to be relatively high in a seed on account of its low water content, so that the dose rate to which an embryo is exposed may well be an order of magnitude greater than that in meristematic tissue of a mature plant. No quantitative estimate of break frequency can, however, be attempted, first because no α-ray data are available for *Allium* chromosomes, and secondly, because the laws governing the production of aberrations in dormant seeds appear to differ from those with which we have become familiar through the study of microspores, root tips, and other tissue, as is evidenced by the observations of Sax (1941) that fractionation of an X-ray dose to dormant seed increases instead of decreases the number of aberrations seen on germination.

Naturally occurring chromosome aberrations can no doubt arise in diverse ways, some of which have been enumerated by Darlington & Upcott (1941). The similarity of some of the aberrations to those produced by X-rays, as well as the circumstances of their occurrences, suggest an ionizing radiation as their immediate causative agent. The evidence appears too fragmentary to make possible a critical assessment of the role of ionizing radiations, but a consideration on the one hand of the wide dissemination in nature of radioactive elements, and on the other of the relatively high efficiency of α-radiation relative to X-radiation in producing chromosome aberrations, particularly the more interesting types involving the union of two separate breaks, focuses attention on the α-rays from the radioactive constituents of living matter and its immediate environment as a possible source of aberrations in circumstances in which the intensity of cosmic radiation and local γ-radiation is clearly insufficient to account for the observed aberration frequency.

SUMMARY

The structural changes seen at metaphase 24 hr. after the irradiation of microspores of *Tradescantia bracteata* by known doses of α-radiation have been studied.

The types of structural change produced resemble those seen at the same interval after γ-ray, X-ray and neutron treatment, and have been classified as chromatid and isochromatid breaks, or chromatid exchanges, following Catcheside, Lea & Thoday. The number of structural changes of all three types increases linearly with the dose. The proportion of structural changes in which fusion of broken chromatid ends is incomplete is considerably greater than observed previously with any other types of radiation.

Even when allowance has been made for this fact the number of structural changes primarily produced by a given dose of α-radiation is estimated to be much larger than would have been anticipated from earlier studies with X-rays and neutrons, and suggests that those α-particles which pass close to the chromatid thread as well as those which pass through it have a significant probability of breaking the thread.

The new experimental data direct attention to α-rays arising from the radioactivity of soil and living tissue as the possible origin of certain types of 'spontaneous' chromosome aberrations.

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REFERENCES

BURKSER, E., BRUN, I. & BRONSTEIN, K. (1927). The radioactivity of plants and the existence of radium element in them. *Biochem. Z.* **181**, 145.

CATCHESIDE, D. G., LEA, D. E. & THODAY, J. M. (1946*a*). Types of chromosome structural change induced by the irradiation of *Tradescantia* microspores. *J. Genet.* **47**, 113.

CATCHESIDE, D. G., LEA, D. E. & THODAY, J. M. (1946*b*). The production of structural changes in *Tradescantia* microspores in relation to dosage, intensity, and temperatures. *J. Genet.* **47**, 137.

DALE, W. M. (1940). The effect of X-rays on enzymes. *Biochem. J.* **34**, 1367.

DALE, W. M. (1942). The effect of X-rays on the conjugated protein amino-acid oxidase. *Biochem. J.* **36**, 80.

DALE, W. M. (1943). Effect of X-rays on aqueous solutions of biologically active compounds. *Brit. J. Radiol.* **16**, 171.

DARLINGTON, C. D. & LA COUR, L. F. (1945). Chromosome breakage and the nucleic acid cycle. *J. Genet.* **46**, 180.

DARLINGTON, C. D. & UPCOTT, M. B. (1941). Spontaneous chromosome change. *J. Genet.* **41**, 297.

EVANS, R. D. & KIPP, A. F. (1938). The radium content of marine sediments from the East Indies, the Philippines, and Japan, and of the mesozoic fossil clays of the East Indies. *Amer. J. Sci.* **36**, 321.

EVANS, R. D., KIPP, A. F. & MOBERG, E. G. (1938). The radium and radon content of Pacific Ocean water, life and sediments. *Amer. J. Sci.* **36**, 241.

FABERGÉ, A. C. (1941). An experiment on chromosome fragmentation in *Tradescantia* by X-rays. *J. Genet.* **43**, 189.

GILES, N. H. (1940). The effect of fast neutrons on the chromosomes of *Tradescantia*. *Proc. nat. Acad. Sci.* **26**, 567–75.

GILES, N. H. (1941). Spontaneous chromosome aberrations in triploid *Tradescantia* hybrids. *Genetics*, **26**, 632–49.

GILES, N. H. (1943). Comparative studies of the cytogenetical effects of neutrons and X-rays. *Genetics*, **28**, 398.

GRAY, L. H., MOTTRAM, J. C., READ, J. & SPEAR, F. G. (1940). Some experiments upon the biological effects of fast neutrons. *Brit. J. Radiol.* **13**, 371.

GRAY, L. H. & READ, J. (1942). The effects of ionizing radiations on the broad bean root. Part IV. The lethal effect of α-radiations. *Brit. J. Radiol.* **15**, 320.

JANITZKY, A., KREBS, A. & RAJEWSKY, B. (1938). On the problem of radium poisoning. *Strahlentherapie*, **61**, 254.

KOLLER, P. C. (1943). The effects of radiation on pollen grain development, differentiation, and germination. *Proc. Roy. Soc. Edinb.* B, **61**, 398.

KREBS, A. (1939). Über die normale und anormale Radioactivität mänchlichen und tierichen Gewebes. *Fund. Radiol.* **5**, 89.

KUNASHEVA, K. G. (1939). Distribution of radium and uranium in plants raised on media with different concentrations of these elements. *Trav. Lab. Biogiochem. Acad. Sci. U.R.S.S.* **5**, 197.

LEA, D. E. (1946). *Action of Radiation on Living Cells.* Camb. Univ. Press.

LEA, D. E. & CATCHESIDE, D. G. (1942). The mechanism of the induction by radiation of chromosome aberrations in *Tradescantia*. *J. Genet.* **44**, 216.

LEA, D. E., SMITH, K. M., HOLMES, B. & MARKHAM, R. (1944). Direct and indirect actions of radiation on viruses and enzymes. *Parasitology*, **36**, 110.

NICHOLS, C. (1941). Spontaneous chromosome aberrations in *Allium*. *Genetics*, **26**, 89.

RUTHERFORD, E. (1913). *Radioactivity.* Camb. Univ. Press.

PELZ, F. M. (1939). *Anz. Akad. Wiss. Wien* (Math. Naturw. Klasse), **117**, 19.

SANDERSON, J. C. (1911). The probable influence of the soil on local atmospheric activity. *Amer. J. Sci.* **32**, 169.

SAX, K. (1938). Chromosome aberrations induced by X-rays. *Genetics*, **23**, 494–516.

SAX, K. (1940). An analysis of X-ray induced chromosomal aberrations in *Tradescantia*. *Genetics*, **25**, 41.

SAX, K. (1941). The behaviour of X-ray induced chromosomal aberrations in *Allium* root tip cells. *Genetics*, **26**, 418.

SAX, K. (1941). Types and frequencies of chromosomal aberrations induced by X-rays. *Cold Spr. Harb. Symp.* **9**, 93.

SIZOO, G. J., SANDERS, P. C., FRIELE, L. F. C. & VAN DER MAAS, G. J. (1941). Measurements on the emanation content of ground air. *Physica*, **8**, 647.

THODAY, J. M. (1942). The effects of ionizing radiations on the chromosomes of *Tradescantia bracteata*. A comparison between neutrons and X-rays. *J. Genet.* **43**, 189.

THOMPSON, F. C. & ROWLANDS, S. (1943). Dual decay of potassium. *Nature, Lond.* **152**, 103.

THE INHERITANCE OF RED, ROAN AND WHITE COAT COLOUR IN DAIRY SHORTHORN CATTLE

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(With Plates 6 and 7)

I. Introduction

In Shorthorns, in the vast majority of cases, both red and white animals breed true for colour and crosses between them give roans.

The simplest explanation is that red and white are allelomorphs, the heterozygote giving roan (Wilson, 1908; Smith, 1925; Roberts, 1937). Some workers, recognizing the fundamental redness of Shorthorns, have suggested theories to meet this. Walther (1913) argued that the colour must be 'a fixed red', the white 'moving over a large area'. He postulated one pair of allelomorphs, therefore, white and the absence of white. Crew (1925) suggested that 'all Shorthorns may be reds...' and looked upon white as due to a modifying factor. Wright (1917), considering the production of some animals with red pigment in the F_2 generation of white Shorthorn by Galloway crosses, postulated two pairs of allelomorphs—**E**, a factor for black allelomorphic with **e**, a factor for red, and **W**, white, allelomorphic with **w**, the absence of white, the heterozygote **Ww** giving roan. Ibsen (1933), also concerned with the Shorthorn by Galloway crosses, postulates two pairs of allelomorphs—**B**, black, allelomorphic with **b**, absence of black and **N**, white, allelomorphic with **n**, absence of white. He further postulates hypostatic red (**R**) homozygous in all cattle.

There are certain exceptions to the general plan of inheritance which appear in all data whether taken from the herd books or collected directly in the field. Roans are occasionally recorded from red × red crosses and from white × white, and both reds and whites occasionally appear from red × white and whites from red × roan. Such anomalous results have been explained in various ways.

Those workers who have postulated theories to account for the general plan of inheritance, as mentioned above, have sometimes dismissed the exceptions as due to errors in recording or in identification (Wilson, 1908; Walther, 1913; Smith, 1925); in other cases they have offered subsidiary hypotheses to account for them. Walther (1913) and Smith (1925) suggest that roaning may be lost with age and so lead to wrong identification. Walther (1913) suggests that the factors themselves may change quantitatively. Ibsen (1933) postulates a roan modifier, **rm**, which is recessive and which changes roan to red in the homozygous condition.

Other workers, accepting at least some exceptions as valid, have attempted to frame theories to embrace the complete data (Laughlin, 1911; Lloyd Jones & Evvard, 1916; Duck, 1923; Evvard, Shearer, Lindstrom & Smith, 1930). All such theories are unsatisfactory in one way or another. As pointed out by other workers, some break down statistically, some demand genotypes which do not exist (Wright, 1917; Crew, 1925; Smith, 1925; Gowen, 1927; Ibsen, 1933). Such theories need not be discussed here because the new facts, given below, show beyond all reasonable doubt that records which *appear*

to be exceptional *are in fact* exceptional, but that they have a very simple explanation which brings them into the general scheme of inheritance.

II. New data

In the present investigation the Duke of Westminster's Eaton Herd of Pedigree Dairy Shorthorn cattle has been studied, both in the field and from the herd books as well as from private records and photographs. The ancestry of the existing herd§ is taken back five generations. Two generations, and often a third, are still in the herd and available for examination; furthermore, the majority of the recorded matings used herein are within the memory of the bailiff, Mr Pakenham Hamilton.

Every combination of colours has been made in the matings and all colour types have been produced, but breeders have a predilection in favour of some colours, and the numbers in these cases are overweighted in the recorded results. Hence the ratios of the colours obtained from the records have no significance.

Reds and red-and-whites are here recorded as such but, for purpose of analysis, the red-and-whites are treated as reds, white spotting being inherited as a separate character (Smith, 1925). Similarly, the roan-and-whites are treated as roans—to avoid confusion they are also recorded as roans, for a small amount of white spotting on a light roan is not easy to identify with certainty.

A summary of the data obtained in the present study is given in Table 1. The sex is not recorded, as reciprocal crosses give exactly similar results.

Table 1

Roan includes roan-and-white.

Type of cross		Results				
		Red	Red and white	Roan	White	Totals
Red	× red	16	12	2‡	—	30
Red	× red-and-white	16	13	4‡	—	33
Red-and-white	× red-and-white	2	12	2‡	—	16
White	× red	1‡	—	27	—	28
White	× red-and-white	—	—	16	—	16
White	× white	—	—	—	1	1
Roan	× red	66	31	126	1‡	224
Roan	× red-and-white	27	52	98	1‡	178
Roan	× roan	28	45	177	47	297
Roan	× white	—	—	21	12	33
Totals		156	165	473	62	856

‡ Unexpected on any monohybrid theory of roan.

Counting red-and-whites as reds and roan-and-whites as roans, the crosses examined yield the results shown in Table 2.

Table 2

Type of cross	Results			
	Red	Roan	White	Totals
Red × red	71	8‡	—	79
White × red	1‡	43	—	44
White × white	—	—	1	1
Roan × red	176	224	2‡	402
Roan × roan	73	177	47	297
Roan × white	—	21	12	33
Totals	321	473	62	856

‡ Unexpected on any monohybrid theory of roan.

§ In 1939.

III. DISCUSSION OF THE GENERAL SCHEME OF INHERITANCE

These data correspond closely with those collected by other workers. Apart from the exceptions to be discussed below, they could be explained by considering red and white as allelomorphs. This theory, however, demands the absence of red in white animals and, as many workers have realized, is unsatisfactory in the light of white Shorthorn by Galloway crosses. Further, it is entirely out of harmony with the observed facts of pigment distribution. White Shorthorns are never without visible red in the coat—about the ears and in the eyelashes—and microscopically the white hairs themselves all show some small amount of red pigment (unpublished data). It seems evident, therefore, that all Shorthorns are red, as Crew (1925) suggested, and the only remaining question is whether their red is the allele of the black of black breeds, as postulated by Wright (1917), or whether red is hypostatic as postulated by Ibsen (1933). From Bogart & Ibsen's observations (1937) and from our own (unpublished), it is certain that homozygous black hair contains red pigment so that black and red cannot be allelomorphs. It seems most probable, therefore, that both black breeds and Shorthorns contain hypostatic red.

The scheme of inheritance therefore which most satisfactorily meets our own observed facts is that put forward by Ibsen in 1933:

R = red—always homozygous.
N = white—causes hair of any colour to become devoid of pigment, incompletely dominant.
n = not white—leaves the hair pigmented.
Thus, red = **RRnn**, roan = **RRNn**, white = **RRNN**.

For the black of Galloways Ibsen postulates a factor **B**, allelomorphic with **b**, which gives absence of black. Thus the formulae for the main colour types involved in the white Shorthorn × Galloway cross will be:

Black	**BBnn** or **Bbnn**	with red (**R**) homozygous in all cases.
Red	**bbnn**	
White	**bbNN**	
Red roan	**bbNn**	
Blue roan	**BBNn** or **BbNn**	

This scheme takes no account of the variations in shade of red in Shorthorns—variations which are of even wider range in cattle as a whole. The constitution of red is by no means fully understood, and the absence of a known allele presents difficulty. But that red is present seems beyond doubt and the postulate of homozygous hypostatic red (**R**) meets the case, though only in the nature of a first approximation.

Ibsen's 1933 theory of colour inheritance was modified by Bogart & Ibsen in 1937 because they believed that they had demonstrated black pigment in red and white hair. Microscopic studies similar to theirs have been made in the course of the present investigation and have entirely failed to reveal any black pigment in either red or white hair of Shorthorns. Furthermore, results exactly similar to theirs, but in our case certainly false, can be obtained readily by certain methods of examination and this, we believe, is the explanation of their findings. We hope to publish the results of our own studies of pigmentation shortly, when the question of both black and red pigment will be more fully discussed.

IV. Exceptions: Analysis and discussion

On the theory here postulated there are eleven unexpected results shown in Table 1:

Red	× red	gave roan in 2 cases
Red	× red-and-white	gave roan in 4 cases
Red-and-white	× red-and-white	gave roan in 2 cases
Red	× white	gave red in 1 case
Red	× roan	gave white in 1 case
Red-and-white	× roan	gave white in 1 case

In view of the possibility of herd-book errors (of the clerical type), the eleven unexpected animals, recorded above, have been examined as far as possible to check the colour. In two cases (1 and 2, below) we have seen the animals themselves, Eaton Waterloo Swell and Woodhull Grey, and there is no doubt that they are correctly registered. In six other cases we have excellent photographs, and in four of these (3, 5, 7 and 9, below) the description is certainly correct—they are described as roans and the photographs show them unmistakably as such. In the other two cases (4 and 8, below) it is almost certain that the registered description is correct, but as one is white and the other red it is not possible to be as certain, from a photograph, as in the case of roans. In only three cases have we been unable to check the registered descriptions (6, 10 and 11, below). The same possibility of clerical errors in the herd-books arises also in regard to the sire and dam of an unexpected animal. Therefore these, too, have been checked as far as possible for colour. In some instances the animals themselves have been examined, in others good photographs have been available, and in all these cases the animals appear to be as registered. In a few instances it has not been possible to check the colour, but there are several cases where sire, dam and offspring have all been sufficiently checked to make it practically certain that in these cases at least there has been no herd-book error of the clerical type (1, 3, 5 and 7, below).

There is always, however, the possibility of mistaken identity. The most striking feature in a Shorthorn herd is the extreme variation in the degree of roaning (Pl. 6, figs. 1–12). Some roans have so little white in the coat that it is easy to mistake them for reds, others are so white that only close examination shows them to be roans, and this must inevitably result in some animals being wrongly described and so misregistered. Wilson (1908), Laughlin (1911), Walther (1913), Wentworth (1913) and Smith (1925) have all noted such cases of wrong description. Furthermore, early in the course of the present investigation, working with the animals in the field, it seemed unlikely that the visible limits of variation are in fact its full limits. If a roan can be so nearly red as to be mistaken for red it seemed probable that they are sometimes just that slight degree more red which would make them truly phenotypically red and, similarly, that occasionally they may be phenotypically white. In that case there should be animals correctly described and registered as reds (or as red-and-whites) and as whites, which are genotypically roan and which therefore breed as roans.

With these possibilities in mind, then, every anomalous case in our data has been carefully examined. The progeny of the unexpected animal itself and of both its sire and dam have been traced in the herd-books wherever possible and in nine of the eleven cases recorded one or other of the three animals under consideration has been found from its

breeding record to be genotypically roan although not registered as such. In the other two cases the necessary data were not available. A detailed analysis of the eleven anomalous cases is given below.

Note. In animals marked * the colour has been checked from the animal itself. In those marked † the colour has been checked from photographs only. The number of an animal (and the volume and page number), refers to *Coates's Shorthorn Herd-Book* in which all British Pedigree Shorthorns are registered, giving, *inter alia*, parentage and colour. In the herd-books all males have numbers by which they can be traced. Until recently, however, females were not numbered, so that in referring to dams of an earlier date it is necessary to give the volume and page for herd-book reference. The number of a male is always placed after the name, the number of a female, if present, is in front of the name. For ease of reference the herd-book practice has been followed in this paper, although it gives a suggestion of inconsistency in nomenclature.

Case 1. Red-and-little-white × roan gave white:

Hill Victor,† 190567 (red-and-little-white)
2980, Haining Waterloo* (roan) } Eaton Waterloo Swell,* 215328 (white)

All the recorded matings of these animals were examined, and it was found that Haining Waterloo, with two additional matings, and Eaton Waterloo Swell, with three recorded matings, were concerned in no exceptional results. Hill Victor, however, gave twenty-eight registered offspring, and of these eight were anomalies if he were red-and-white. All would be normal if he were roan, except one which is equally anomalous whether he were roan or red-and-white: mated to a white (320022 Pearl Lady) he gave a red-and-little-white (92995 Kidside Pearl). Pearl Lady gave only one other registered offspring and this was unexceptional. There are no recorded offspring of Kidside Pearl so that no light can be thrown on her constitution.

Case 2. Red × red gave roan:

Ireby Warrior,† 223955 (red)
87148, Wreay Grey 12th (red) } 122976, Woodhull Grey* (roan)

Only one other mating of Wreay Grey 12th could be traced and four of Woodhull Grey. All gave unexceptional results. Ireby Warrior, however, gave two anomalies in nineteen matings. Seventeen of these matings were of such a type that the recorded results were equally to be expected whether he was roan or red, but in the other two, which alone were specific, he acted as a roan.

Case 3. Red × red gave roan:

Eaton Stirling Prince,† 180549 (red)
8982, Eaton Princess Gift† (red) } 79178, Woodhull Princess Gift† (dark roan)

Woodhull Princess Gift gave six calves, Eaton Princess Gift gave an additional twelve; none of these were anomalous. Eaton Stirling Prince produced twenty-six offspring, twenty-four of which were unexceptional whether he was red or roan. The remaining two were normal if he were roan, anomalous if he were red—mated to a red he gave roan, mated to a white he gave a white.

Case 4. Red × roan gave white:

Kelmscott Acrobat 4th, 126217 (red)
Orange 52nd,† V. 64, p. 993 (roan) } Kelmscott Conjurer 56th,† 181977 (white)

Kelmscott Conjurer 56th gave no exceptions in fourteen matings and Orange 52nd, in five other matings which were traced, gave only normal results. Kelmscott Acrobat 4th gave 126 recorded offspring, but in 116 matings only could the colour of the dam be found. If he were a red, as registered, then eight of the 116 matings produced anomalous offspring. All were normal if he were roan. In the remaining 108 he could have been either roan or red.

Case 5. Red × red-and-white gave roan:

Lawnhead Cavalier,† 182413 (red) 43883, Duchess of Ranton 3rd† (red-and-white)	438336, Duchess of Ranton 6th† (red roan)

No offspring of Duchess of Ranton 6th could be traced. Lawnhead Cavalier gave no other exceptions in eight recorded matings. Duchess of Ranton 3rd, however, produced four offspring, two of which were anomalous if she were a red-and-white as registered, but all would be normal if she were a roan.

Case 6. Red × red-and-white gave roan:

Vestris Duke, 113607 (red) Rocks Cup,† V. 58, p. 439 (red-and-white)	Rosamund of Chesterton 2nd, V. 61, p. 642 (roan)

No offspring of Rosamund of Chesterton 2nd could be traced. Vestris Duke gave nine additional recorded offspring, all of which were unexceptional. Rocks Cup, however, with four recorded offspring, produced two which were anomalous in colour if she were red-and-white but all would be normal if she were roan.

Case 7. Red × red-and-little-white gave roan:

Longhills Count,† 116262 (red) Dolphinlee Flora,† V. 58, p. 683 (red-and-little-white)	Preshute Dolphin, 127358† (dark roan)

Dolphinlee Flora gave two exceptional results in seven matings. All would be normal if she were roan. Preshute Dolphin produced nineteen offspring, none of which were anomalous. Only one other mating of Longhills Count could be traced, this being unexceptional.

Case 8. Red × white gave red:

Bessie's Champion,† 178498 (white) Duchess 178th, V. 61, p. 767 (red)	37090, Duchess 210th† (red)

Only one other mating of Bessie's Champion could be traced, and the result of this was unexceptional. Duchess 178th gave three other recorded offspring all of which were normal. Duchess 210th, however, gave four offspring, two of which were anomalous if she were red, as registered, but all would be normal if she were roan.

Case 9. Red-and-white × red gave roan:

Upton Lord Waterloo 2nd, 146048 (red-and-white) Kenilworth Dairymaid, V. 64, p. 740 (red)	Kenilworth Dairymaid 2nd,† V. 64, p. 740 (roan)

Of the four matings of Kenilworth Dairymaid, two produced anomalous offspring and indicated she was breeding as a roan. Kenilworth Dairymaid 2nd gave six unexceptional offspring, breeding according to her registered colour. No other matings of Upton Lord Waterloo 2nd could be traced.

Case 10. Red-and-little-white × red-and-little-white gave roan:

Snowdon Boy, 145575 (red-and-little-white) Queen of France, V. 60, p. 1058 (red-and-little-white)	Queen of the West, V. 67, p. 526 (dark roan)

Queen of France gave five other offspring, Queen of the West gave two, and all were unexceptional. No further matings of Snowdon Boy could be traced.

Case 11. Red-and-white × red-and-little-white gave roan:

Plas Power General, 112799 (red-and-white) Wild Eyebright 11th, V. 56, p. 1135 (red-and-little-white)	Plas Power Eyebright, V. 61, p. 736 (dark roan)

Wild Eyebright 11th gave three other offspring and Plas Power Eyebright gave seven offspring, all of which were unexceptional. No further matings of Plas Power General could be traced.

Thus in nine of the eleven anomalous cases examined above the unexpected animal itself or its sire or its dam has been found to be breeding as roan though registered as red or as red-and-white, so that the anomalies depend simply upon the mistaken identity of certain genotypic roans. In the two remaining cases the necessary data are not available.

In the present data there is an unusually low percentage of exceptions in the records—only 1·2 % as compared with an average of 5·8 % (Smith, 1925, consolidated data), and this is probably due to the fact that one particular herd has been studied which is in the hands of an exceptionally careful recorder (Mr Hamilton). Even so there is one animal in the present herd which is known to have been wrongly described, Eaton Winsonia 13th, V. 87, p. 873, registered as a red-and-white was later found to be a dark roan. And perhaps some of the nine animals under discussion may similarly have been wrongly described. Eaton Stirling Prince, however (Case 3), is almost certainly correctly described as red. Mr Hamilton knew him well; he was in the Eaton Herd for many years and was a noted champion. It is practically impossible that he was wrongly registered. Walther (1913) records two similar cases, and these three appear to be examples of animals which though genotypically roan, are phenotypically truly red.

There are no cases in our data of genotypic roans being registered as whites, but from the literature it is evident that such cases do occur. Wentworth (1913) reports one case of wrong description, a very light roan which was registered as a white. And Walther (1913) records one white which, on most careful inquiry from the breeder, seemed to be really white yet bred as a roan.

So that at the white end of the roaning series, as well as at the red, roan seems to pass imperceptibly into self-colour, and this is probably the explanation of most of the exceptions on record.

It is doubtful whether it is the full explanation of the much-discussed white bulls, which, mated to red cows, have given a considerable proportion of red offspring. Laughlin (1911) records one such bull which, mated to reds, gave forty-one roans, nine reds and four red-and-whites; mated to a roan he gave one white. Lloyd Jones & Evvard (1916) record a white bull, Whitehall Sultan, 163573,‡ which, mated to reds, gave forty-four roans and fifteen reds. Smith (1925), quoting from the unpublished data of Martini, records another white bull, Maxwalton Sultan, 305870,‡ son of Whitehall Sultan, which, mated to reds, gave forty-one roans, thirteen reds and two whites. Smith, pointing out

‡ American Herd-Books.

the difficulty, which sometimes occurs, of distinguishing a red roan from a red, suggests that the thirteen reds were probably red roans, and that the two whites were herd-book errors or mutations. His suggestion of wrong description demands an error in registration of about 25 %, yet he states that Martini had sought for cases of misregistration among the offspring of Maxwalton Sultan but had found none. Ibsen (1933), referring to the cases of Whitehall Sultan and Maxwalton Sultan, postulates, in Shorthorns, a roan modifier, **rm**, a rare recessive which, when present in the homozygous condition, changes roan to red. But Whitehall Sultan, if he were homozygous for **rm**, must have met at least fifteen cows homozygous for **rm**—if he were heterozygous for the factor he must have met something like thirty homozygous for **rm**—out of fifty-nine. If he were heterozygous for **rm** and the cows heterozygous, then practically all must have carried it to give fifteen **rmrm** in fifty-nine matings. Yet Ibsen suggests it as a rare recessive. If it were as common as it would have to be to give the results recorded for Whitehall Sultan and Maxwalton Sultan its incidence would give results in the general population entirely at variance with the observed facts of inheritance; for, however it may be interpreted, red × white gives roan in an overwhelmingly high proportion of cases. It seems more probable that Whitehall Sultan and Maxwalton Sultan and other similar anomalous white bulls referred to in the literature were, in fact, genotypic roans which were phenotypically at the extreme white end of the series. (The two whites among the offspring of Maxwalton Sultan would still be exceptional even though he were breeding as a roan—unless both they and he were extremely white roans.) The only difficulty in accepting this explanation is that, where the details of the progeny are recorded, the proportion of roans to reds is very high. The numbers are small and may have no significance but the results in the three cases are surprisingly similar.

V. Grades of roaning

There remains for discussion the possible causes behind the various grades of roaning. White spotting may sometimes add to the whiteness of a roan, but it could never seriously mask the degree of roaning. In the Eaton Herd, at least, white spotting is mainly ventral in distribution, so that when present to its maximum extent it does not involve the dorsal half of the animal except sometimes across the shoulders and the forehead. So that the grade of roaning of an animal can be judged quite apart from any consideration of the areas subject to white spotting.

Wright (1917) suggested that the various grades might be given by a series of multiple allelomorphs, producing different strengths of white. That this is not the case is shown by the fact that a roan, mated to reds, gives *many* different grades of roan. One example is shown in Pl. 7, figs. 13–16. The sire, Eaton Rose King, 207056 (fig. 13), mated to reds gave red (fig. 14), medium roan (fig. 15) and light roan (fig. 16), as well as many intermediate grades. Clearly this is not a case of multiple allelomorphs.

Crew (1925) suggested that possibly there are modifying factors. There is no evidence for specific modifying genes and until such evidence is forthcoming it seems better not to postulate them. It appears that, in different internal environments, red and white express themselves in varying degrees—beyond that it seems unsafe to go.

Fig 1.

Fig 2.

Fig. 3

Fig. 4.

Fig. 5.

Fig 6.

Fig 7.

Fig 8.

Fig 9

Fig. 10.

Fig 11.

Fig 12.

PLATE 6

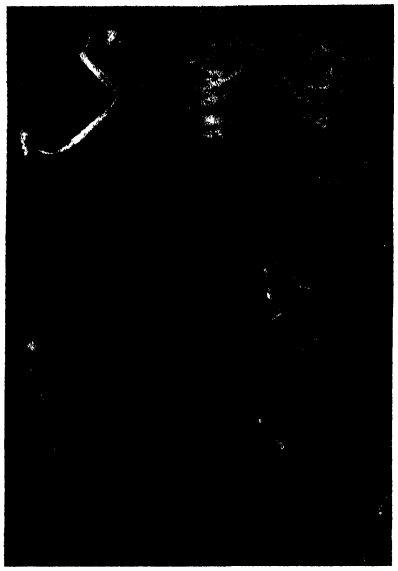

Fig. 14

Fig. 16

Fig. 13

Fig. 15.

SUMMARY

1. Inheritance of red, white and roan in Shorthorn cattle is best explained on Ibsen's 1933 theory that red (**R**) is hypostatic and homozygous in all Shorthorns, and that white is due to a factor (**N**) which in the heterozygote gives roan.

2. Recorded exceptions to the main plan of inheritance are explained by the fact that genotypic roan extends from complete red to complete white in the phenotype.

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REFERENCES

BOGART, R. & IBSEN, H. L. (1937). The relation of hair and skin pigmentation to colour in cattle, with some notes on guinea-pig hair pigmentation. *J. Genet.* **35**, 31–60.

CREW, F. A. E. (1925). *Animal Genetics.* Edinburgh.

DUCK, R. W. (1923). Colours of Shorthorn cattle. *J. Hered.* **14**, 65–75.

EVVARD, J. M., SHEARER, P. S., LINDSTROM, E. W. & SMITH, A. D. B. (1930). The inheritance of colour and horns in blue-gray cattle. 2. *Res. Bull. Ia. Agric. Exp. Sta.* no. 133, pp. 1–16.

GOWEN, J. W. (1927). A resumé of cattle inheritance. *Bibliogr. genet.* **3**, 87–137.

IBSEN, H. L. (1933). Cattle inheritance. I. Colour. *Genetics*, **18**, 441–80.

LAUGHLIN, H. H. (1911, 1912). The inheritance of colour and horns in Shorthorn cattle. *Amer. Nat.* **45**, 705–42 and **46**, 5–28.

LLOYD JONES, O. & EVVARD, J. (1916). Inheritance of colour and horns in blue-gray cattle. *Res. Bull. Ia. Agric. Exp. Sta.* no. 30, pp. 67*a*–106*a*.

ROBERTS, E. (1937). Colour inheritance in Shorthorn cattle. *J. Hered.* **28**, 167–8.

SMITH, A. D. B. (1925). Shorthorn colours. *J. Hered.* **16**, 73–84.

WALTHER, A. R. (1913). Die Verebung unpigmentierter Haare (Schimmelung) und Hautstellen ('abzeichen') bei Rind und Pferd als Beispeile transgressiv flucturiender Faktoren. *Z. indukt Abstamm.-u. VererbLehre*, **10**, 1–48.

WENTWORTH, E. N. (1913). Colour in Shorthorn cattle. *Amer. Breed. Mag.* **4**, 202–8.

WILSON, J. (1908). Mendelian characters among Shorthorn cattle. *Sci. Proc. Dublin Soc.* **11** (N.S.), 317–24.

WRIGHT, S. (1917). Colour inheritance in mammals. VI. Cattle. *J. Hered.* **8**, 521–7.

EXPLANATION OF PLATES 6 AND 7

PLATE 6

Figs. 1–12. Photographs showing different grades of roaning, from dark roan (fig. 1) to light roan (fig. 12).

PLATE 7

Photographs showing Eaton Rose King, 207056, and three of his offspring from red cows.

Fig. 13. Eaton Rose King, 207056, roan.
Fig. 14. Red.
Fig. 15. Medium roan.
Fig. 16. Light roan.

THE PROBLEM OF NON-HEREDITARY ADAPTIVE MODIFICATIONS (COINCIDENT OR ORGANIC SELECTION)

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Recent years witnessed the rise of renewed interest in the question of the interrelation of non-hereditary adaptive modifications and natural selection. Among the large number of papers published on this subject, those printed in Russian occupy a significant place. The problems discussed by the Russian scientists are of considerable importance in clarifying the theory of evolution. A review of these papers may be of general interest. In this connexion it may be in place to give a brief outline of the development of the problems concerned.

I. The hypothesis of coincident and organic selection

Towards the end of the last century Lloyd Morgan (1896), Osborn (1896, 1897), and Baldwin (1896,* 1902) simultaneously and independently of one another proposed a new hypothesis of the selection of coincident variations or, as Osborn and Baldwin termed it, of organic selection. This hypothesis was supported by Poulton, Wallace, Gulick, and can be summarized as follows:

Individual plasticity is the property of all organisms. According to Morgan and Baldwin plasticity is acquired in the struggle for life as the result of natural selection. According to Osborn it is an inherent quality of living matter, the property of protoplasm. Osborn found it impossible to explain in any other way the experimentally established ability for adaptation to conditions of life not only unusual for the existing forms, but for their ancestors as well.

The plasticity of organisms is expressed in the form of change in habits and of morphological adaptive alterations. Habit alters the structure of the organism including such basic organs as the skeleton, the circulatory system, etc.

If individual adaptive modifications happen to be present, those among the numerous divers congenital variations will be favoured that are coincident with a given useful modification. Such variations will gradually accumulate. If the conditions that called forth the given plastic modification are continued for a considerable period, the accumulation of coincident variations will progress slowly, but inevitably.

According to Lloyd Morgan and Baldwin this accumulation is the result of the extinction of all variations going in other directions and the preservation of all coincident variations, since they enhance non-hereditary change and supplement its shortcomings. From Osborn's point of view the accumulation of hereditary variations similar to modifications has nothing to do with selection, but takes place automatically since all other variations are eliminated.

The plastic (phenotypical) adaptations will be substituted by hereditary ones. Non-hereditary alterations of habit and structure acquire phylogenetic significance. As an

* Cit. after Baldwin (1902).

illustration Lloyd Morgan refers to alterations that take place in bone structure. Certain individuals reveal an increased growth of bone structure as the result of increased function and of the influence of contiguous parts in an altered environment and with the acquisition of new habits. This may be qualified as a plastic modification. After such an alteration congenital variations leading also to an intensified bone development will no longer be eliminated. Moreover they will also be accumulated due to their usefulness since they enhance the plastic modification. The accumulating hereditary variations will finally effect a similar strengthening of the bone structure which had been hitherto the result of habitual use, that is, had been due to the plasticity of the organism.

The fixation of plastic modifications does not imply the direct inheritance of acquired characters and the results of the exercise or desuetude of organs. It should be attributed to the automatic accumulation or selection of coincident variations, and it satisfactorily explains the parallelism between hereditary and non-hereditary variability which is often observed in the case of many animals and plants. Osborn and Lloyd Morgan emphatically stress the point that their hypothesis undermines the Lamarckian principle of evolution and destroys its basic arguments.

Baldwin points to the fact that organic selection not only stabilizes modifications but further expands plasticity in the same direction (see also Gulick, 1905). According to Baldwin organic selection attains first rate importance in evolution because of the existence of broad and complex accommodations of a general nature. Detto (1904) likewise refers to such modifications, indicating that selection creates comparatively non-specific reaction systems which account for the existence of a 'modification reserve' (see also Lukin, 1942). Thus Osborn's 'self-adaptation' in an unusual environment can be easily explained without resorting to the inherent plasticity of the protoplasm.

Gulick (1905) likewise supports the hypothesis of the selection of coincident variations and calls it 'coincident selection'. He particularly stresses the significance of the isolating influence of modifications which hamper free cross-breeding. Lately, this particular role of modifications has been discussed in works on ecological isolation among birds (Promptov, 1934*a*,*b*, 1936; Hogben, 1940), on biological races of insects (Thorpe, 1940), races of fish (Kirpichnikov, 1933; L. S. Berg, 1934) and many others. Muller (1942) has analysed the question in detail and illustrated his arguments with examples of temperature modifications in *Drosophila*.

Huxley (1942) cites numerous cases in which there is a non-hereditary barrier for cross-breeding at the first stage of divergent evolution.

The hypothesis of coincident selection proposed by the early American authors was gradually forgotten in the years that followed. Baur (1922) and other well-known biologists talk of individual plasticity only as the result of natural selection. Turesson (1922) concludes that non-hereditary differences are substituted at the very earliest stages of the formation of new races and ecotypes by similar hereditary changes. Thellung (1930) refers to a corresponding replacement, and explains it by the fact that hereditary variations are more specialized and require less energy for the development of the character. All these remarks are of a casual nature and are not in any way connected with the views of Lloyd Morgan and his contemporaries. Only in 1935 did this problem once more become the object of discussion, particularly in Russian works. In his latest very interesting book Huxley (1942) lays much stress on the principle of Baldwin and Lloyd Morgan's organic selection.

II. 'Indirect selection' and the fixation of modifications according to Kirpichnikov

In a paper on the problem of race-formation in fishes (Kirpichnikov, 1933) and especially in succeeeding papers (1935, 1940) an attempt is made to give a general analysis of the origin and the significance of non-hereditary adaptive modifications in the formation of species and in evolution (the hypothesis of 'indirect selection').

The capacity to produce adaptive modifications* is the result of selection of viability in a fluctuating environment, in other words the selection of plasticity, which takes place among all organisms. Thus, the ability of plants to alter the length of their roots, the number and size of stomata, and so forth, depending upon the moisture of soil and air, is historically the result of adaptation by means of selection to an ever-changing environment. These modifications spread throughout an entire population and may be repeated for a number of generations.

Not only are constant morphological changes often present and play an important role, being particularly essential to immobile plants (see also Schmalhausen, 1941), but small regulatory, functional and morphogenetic ones also. In higher forms of animals, habits (conditional reflexes) become particularly significant (see, for instance, Krushinsky, 1944, 1946).

'Growth' modifications in fishes serve as an excellent illustration of how completely one modification can effect an organism. Fluctuations in water temperature as well as in the quantity of food in the water lead to serious changes in the rate of growth and differentiation. This leads to changes in the size of the fish at maturity and in the final stages of growth. Changes in the interrelations between the rate of growth and differentiation also influence the process of maturation itself, the quantity of sexual products, the form and proportions of the body. Finally, the number of 'quantitative' characters is changed (Hubbs, 1926, 1940; Kirpichnikov, 1933, 1940, 1943, 1945; Gabriel, 1944, etc.). Such an all-round reaction favours adaptivity to new conditions and opens the road for future adaptation to changing environment.

The existing parallelism of hereditary (systematic) and non-hereditary variability in nature points to the significance of the latter in speciation. There are many cases when closely related species and subspecies of plants and animals differ inherently from one another by characters that are easily altered by external conditions.

The stabilization of non-hereditary variations in speciation is the result of selection, and not due to the direct inheritance of acquired characters. The relation between the organism and its environment, which is unbalanced when the latter is altered, becomes readjusted through selection. Thus 'the curves of adaptivity' are moved in the direction of the changing environment. These curves express the alterations in viability of the individual observed when there are fluctuations in certain ecological factors in nature.

Selection in this case is most intensive when it concerns the less plastic but the most vital structures of the organism. Organs or characters that can be most readily altered are least subject to selection. Nevertheless the course of their development and their correlation with other characters changes more or less through selection. As a result their capacity to adapt themselves to former conditions, which have by this time become rare, disappears altogether.

This gradual destruction of a part of the original adaptability signifies the fixation of

* Further on in the text this capacity will be termed 'adaptability'.

non-hereditary differences. The cause of such fixation lies in the disintegration of useless reaction systems and correlations in ontogeny, through the selection of viability in an altered environment. This disintegration is not the result of an automatic accumulation of mutations that are now uncontrolled by selection as Schmalhausen (1938, etc.) supposes, but is due to the selection of favourable mutations which pleiotropically destroy unnecessary correlations.

Xerophilous plants may serve as an example. Let us assume that a change in the number and structure of the stomata corresponds to the new conditions of a lowered humidity. However, like all adaptive modifications, these alterations are not perfect. Selection will continue to change the structure of the plant: its leaves and roots, the intensity of metabolism, the biochemical constants of its plasm, etc. The entire development of the plant will be altered, so that when the former conditions of humidity return, the restoration of the original structure and number of stomata will be impossible. The longer the selection progresses in the new environment the more probable will be such partial fixation of the adaptive modification.

According to Tachtadgjan (1941) reversions in plants never lead to the complete restoration of the original ancestral form. The reversion results in a structure similar to those of homologous organs of existing plants. Thus, the carpels of several species when reversions take place do not resemble the leaves from which they originated, but they are like the leaves of the existing form which differ considerably from the original type. Each organ undergoes a hidden 'latent' evolution paralleling the easily noted phenotypical evolution. This 'latent' evolution is based upon correlations in the individual development of homologous organs. Tachtadgjan's conclusions are an illustration of the possible means of fixation of non-hereditary differences through selection when there is a correlated change of several organs. Latent evolution does not only alter the 'hereditary base' as Tachtadgjan postulates, but it also leads to changes in development, in morphogenetic conformities, and, therefore, to alterations in the adaptability as well; the result of this process will be the gradual fixation of the modification.

All this concerns only the adaptive *differences* between diverging groups and not the adaptive *characters* themselves. In a changing but not as yet stabilized environment the diversities may become hereditary, but the capacity for modification will not necessarily be narrowed, it may only be transformed. The dependence of development upon external conditions will not necessarily be lessened. In other words, the partial or complete fixation of the differences between populations is possible: (1) when the fluctuations in environment are increased; (2) when the same degree of fluctuations in the natural conditions is preserved even though the environment changes; and (3) when the fluctuations of the environment are decreased. The latter condition acts, especially when a species disintegrates into smaller and more or less isolated populations.

According to the hypothesis of 'indirect' selection the process of adaptation may often be divided into two stages. The first stage leads to the intensification of an adaptability through selection, and the second to the perfection and fixation of the non-hereditary adaptive differences as a result of further selection.

'Indirect' selection in the sense of Kirpichnikov is the selection of variations which are more or less similar phenotypically to adaptive modifications. The modified organ alters by selection very slowly. In other words we really deal in this case with coincident selection. The author therefore is of the opinion that it would be appropriate to substitute

the term 'indirect selection' by the older term '*coincident selection*' the more so since Darwin (1859), Schmalhausen (1938) and many others use the word 'indirect selection' to signify correlated changes (by means of selection) of favourable and useless characters connected with each other in development.

III. The replacement of non-hereditary changes by hereditary ones in evolution according to Lukin, Gause and Waddington

Similarly to Turesson (1922) and particularly Thellung (1930) Lukin (1935, 1936) supposes that adaptive modifications are rapidly substituted by phenotypically similar mutations. This substitution is due to the greater selective value of the latter. Lukin (1935, 1936; see also 1939, 1940, 1942) notes as such the following: (1) the readiness of an organ at the time of the beginning of its function and its complete formation at a given time, (2) the independence of development from accidental fluctuation in the environment, and (3) a greater specialization of hereditary adaptations. Lukin finds that this conception is supported by the early appearance of hereditary differences between populations in natural conditions (1939, 1940) and by the progressive automatization of individual development. Lukin makes no difference between the two phenomena observed in nature, namely: (1) the fixation of diversities or the elimination of the possibility of returning to the original state without compulsory automatization of development (coincident selection), and (2) the fixation of the character, the diminishing of its dependence upon the environment, its stabilization, and the growth of the role played by the autoregulatory processes in development (stabilizing selection).

In his latest paper Lukin (1942) suggests a classification of various types of adaptive modifications. Of particular interest are those modifications that are not adapted to the conditions responsible for their appearance, but happen to be important links in development. Such are the alterations in the sexual glands of animals, the development of which depends upon light, temperature, the landscape, and the presence of individuals of the opposite sex (Mashkovzeff, 1940; Svetozarov & Straich, 1941). Such are the manifold changes in the stage development of plants, diapause phenomena and many other adaptations. As a good example Lukin points to the development of parasitic worms that have two hosts. The completion of their life-cycle depends upon the succession of sharply contrasting conditions.

In the same paper the author discusses the possibility of the substitution of non-hereditary variations by hereditary ones. Modifications adapted to periodically fluctuating factors of the environment are seldom substituted by mutations. The preservation of such lability is also displayed by the already mentioned basic alterations in development, which depend upon external influences. A twofold interaction with the environment, when the active and the selective factors do not coincide, is usually extremely valuable and represents one of the most perfect forms of adaptability.

In fact only those alterations are substituted which are the result of a constant or one sided change in the environment accompanied by the stabilization of the latter.

Gause (1940*a*, 1941*a*), in general agreement with Lukin, discusses the question of the replacement of adaptive modifications by mutations in the course of selection. He supports his views by the results of a number of experiments on selection among Infusoria. He calls this selection substituting or stabilizing selection (Gause, 1941*a*; Gause, Smaragdova & Alpatov, 1942). Gause notes that in his and Smaragdova's (1940) experiments there was

not a complete phenotypical likeness between adaptive modifications and mutations, and that their similarity is only partial. Later Gause (1941 *b*) discusses the problem of ecological adaptability and classifies these phenomena from the physiological point of view.

Discussing the same problems in regard to acquired and inherent calluses Waddington (1942) introduced the new idea of the canalization of individual development. The ability to react develops under the controlling influence of natural selection. In later stages, as a result of further selection, this ability becomes canalized. This signifies that development may progress only in one or a few directions due to the regulation against accidental deviations and the perfection of threshold reactions. External stimuli act as 'arrows'; the differentiation of the intermediate type becomes impossible. The selective value of the complete independence of the end product from the environmental fluctuations during the development of an organ finally leads to the substitution of such 'arrow' reagents by genetical ones when the latter begin to act earlier in the development.

IV. The views of Schmalhausen, Muretov and Mashkovzeff

Schmalhausen (1938, 1939, 1940, 1941) mentions three means of hereditary fixation of non-hereditary diversities: (1) fixation in the process of 'indirect' or coincident selection (Kirpichnikov, 1935) which proceeds very slowly since favourable mutations on which this selection is based are very rare, (2) fixation due to the uncontrolled accumulation of numerous genic variations, which destroys the ability of those adaptive reactions which are no longer supported by selection; such an automatic destruction of the unutilized lability progresses with greater rapidity since it is connected with the accumulation of numerous unfavourable mutations, and (3) fixation in the process of the all-significant 'stabilizing' selection. This is the most rapid and the most significant means of fixation in evolution.

The theory of stabilizing selection (see also Schmalhausen, 1946) is deduced from the assumption of a high rate of mutations in nature and of their unfavourable character. The preservation of the existing adaptive normal phenotype is possible only on condition that the mechanisms of heredity and development are continuously readjusted.

Selection tends to preserve the normal type despite a high mutability which denotes that new genetical combinations are tested and that the most stable survive. Development thus changes and becomes less dependent upon external influences, although the phenotype of the mature form is not altered.

Such selection implies the general growth of the stability of development, the perfection of various defensive reactions with the growth of autoregulation. Regulatory processes become characteristic for development in general, particularly in higher animals. Reactions connected with the moulding of organs become 'threshold' ones (Goldschmidt, 1938). The distance between the extremes within which the given reaction becomes possible, is increased (Filatov, 1939). Thus development becomes better protected against all kinds of violations (Schmalhausen, 1938, etc.; Muretov, 1941; Kamshilov, 1941; Waddington, 1942). The direct mechanical influence of adjacent parts upon one another (Filatov, 1941) may acquire regulatory significance. The same may be said of processes connected with Child's physiological gradient (Kamshilov, 1941) and the various physiological systems which provide for the adjustment of functions (Kirpichnikov, 1940). Regulatory phenomena, particularly the intensification of sensitivity of organisms (Kalabuchov, 1940 *a*, *b*) compensate their seeming emancipation from the environment.

Stabilizing selection leads to the decrease of variability due to the regulation of all kinds of minor changes and the growth of the influence of internal factors in development. The idea of correlated evolution of such principal properties as the stability of development, adaptability, mutability and dominance were concisely postulated by R. Berg (1944).

Stabilizing selection according to Schmalhausen (1938, etc.) is the principal integrating factor of evolution perfecting development and establishing morphogenetical correlations. Stabilizing selection acts in all instances, notwithstanding the absence or presence of adaptive modifications. In case the last make themselves felt in the processes, stabilizing selection gradually secures the structure of the organ which had been modified. The mechanism of fixation in this case is reduced to the accumulation of mutations and their combinations within the limits of the new adaptive phenotype. The genotype and the development of the organism are likewise reconstructed in correspondence to the new phenotype. In other words, external factors of development are gradually substituted by internal ones. As a result a new and better adapted phenotype is obtained with a maximally stable and independent development.

The substance of the theory of stabilizing natural selection in contrast to 'direct' natural selection is that the former takes place on the basis of selective advantage of the normal phenotype in comparison with the deviations (Schmalhausen, 1941, p. 315). According to Schmalhausen, the selection of favourable deviations from the normal type does not occur in this case. Since, however, Schmalhausen (1941) agrees that the development of the organism is perfected and the role of regulation in its development is increased as a result of which a better adapted phenotype is achieved in the above-mentioned case, the author of these lines does not see the necessity of drawing a strict line between the two conceptions (see Kirpichnikov, 1944*b*).

Experiments on *Drosophila* have shown that small harmful physiological mutations are extremely frequent (Dobzhansky & Queal, 1938; Muretov, 1939, 1941). Muretov speaks of the selection of viability which continually introduces manifold minor genic variations into the normal genotype without a noticeable change in the phenotype. Only the amplitude of reaction in the case of a number of characters is changed. If adaptive modifications happen to be present they become fixed. Thus Muretov's point of view is very similar to that of Schmalhausen's.

The principal evidences in favour of stabilizing selection are the following (Schmalhausen, 1941): (1) progressive development of regulatory mechanisms in evolution, (2) the parallelism of hereditary and non-hereditary variability, (3) incomplete 'reversion' of former characters when the original conditions return, that is partial inheritance of differences between the lower taxonomic categories, (4) the success in elimination of harmful effects of mutations in laboratory strains due to the selection of the most viable combinations of genes, (5) the constancy of the 'wild type' in natural populations in spite of a high mutability, (6) the dominance of the 'wild type', that is the regulation of heterozygous mutations as a partial result of the stabilization of development, and (7) direct experiments on selection carried out by Gause.

Both Schmalhausen (1941) and Lukin (1942) consider the perfection of regulatory mechanisms in the course of evolution due to which development becomes more autonomous and external factors become substituted by internal ones, as an important proof of the correctness of their point of view. Mashkovzeff's (1936) experiments illustrated this

substitution. He compared the ontogenesis and the role of function in the development of nephric ducts, gills and lungs in various representatives of vertebrates.

In agreement with Roux (1881) Mashkovzeff distinguished three stages in the evolution of ontogenesis. In the first stage the leading role in development should be attributed to the environment and to the function of organs. Secondly, the role of function is decreased, the influences of chemical factors and hormones in the embryo itself predominate; the earliest periods of development depend upon inner, hereditary stimulants (all vertebrates including mammals). The third stage is that of labile determination and self-differentiation characterized by complete autonomy of morphogenesis; the development of organs is achieved without the participation of function (partly birds, mostly insects).

The gradually decreasing morphogenic role of function in the course of evolution and, thus, the stabilization of development, as shown by Mashkovzeff's experiments, cannot serve as evidence of the action of stabilizing selection in favour of the fixation of adaptive modifications. These facts as well as some of the points made by Schmalhausen only prove the existence of an important evolutionary tendency towards progressive stabilization of development. In order to verify these conceptions it is necessary to prove them by experiments in selection.

V. THE EXPERIMENTAL CONFIRMATION OF THE HYPOTHESES OF COINCIDENT AND STABILIZING SELECTION

(1) It is a well established fact that the mutability of laboratory strains of *Drosophila* is decreased and variability is in general lowered, whereas the dominance of the normal phenotype is increased. These phenomena are probably the result of selection in conditions of great isolation (Berg, 1942*a*). To this the author would add the consideration that laboratory conditions are characterized by maximum stability, in which variability loses its adaptive significance; besides which it is decreased by the interference of man. In this case stabilizing selection can take place.

(2) The weakening and elimination of the harmful effects of mutations when they arise in laboratory conditions (Fisher, 1928; Schmalhausen, 1938 and others) can also be the result of stabilizing selection.

(3) The experiments of Kamshilov (1939) on the increase and decrease of the effectiveness of the mutation 'eyeless' in *Drosophila melanogaster* permit the conclusion that the selection of individuals with the most notable development of characters results at the same time in the latter becoming more independent of the environment. In other words, the degree of dependence of the development of an organ upon fluctuations of the elements of environment (in this case upon the temperature changes) may be easily modified by means of selection. At the same time these experiments indicate a correlation between the degree of development of characters and their stability.

Indirect data of a similar kind may be found in the work of Berg (1942*b*, 1945) on the correlated differences in mutability, non-hereditary variability and dominance among related populations of *Drosophila*. All these properties determine the general stability of development, i.e. the extent of dependence upon the environment.

(4) Applying X-rays to a large number of *Drosophila*, Naumenko (1941) obtained non-hereditary changes in the wings of all individuals. By selecting the individuals with the most clearly expressed modifications, strains with the same, but already hereditary

changes were obtained. Such artificial stabilizing selection shows the possibility of a similar stabilization in nature.

(5) Smaragdova & Gause (1939) and Gause (1940*b*) successfully subjected the Infusoria *Paramaecium caudatum* and *Euplotes vannus* to a series of experiments with selection in conditions of different salinity. The original cultures adapted themselves relatively quickly to increasing salinity by means of individual adaptability.

Unfortunately, there was no analysis of the adaptive characters themselves in these experiments. The later works of the same authors with *Paramaecium bursaria* are more illustrative. Gause (1941*a*) has shown the adaptive value of decrease of size in Infusoria in high temperature as well as the opposite process in case of low temperature. Smaragdova (1941) found a similar hereditary adaptive geographical variation.

In experiments where selection was practised (Smaragdova, 1940; Gause, Smaragdova & Alpatov, 1942) a hereditary increase and decrease in size was obtained. This change at first appeared—true, in a lesser degree—as a non-hereditary modification. At the same time a new adaptive character—a narrow body—was likewise obtained. These are the first experiments that prove the existence of coincident selection. The authors for some reason call it stabilizing selection, although the adaptability in the strains obtained was not less than in the original ones.

Such are the scant experimental materials. Meagreness of investigations is explained by the difficulty of carrying out exact experiments on selection. There is no doubt that further experiments will follow in the future.

VI. Summary. The significance of adaptive modifications in evolution

Let us attempt to outline briefly the mechanism of coincident selection without any further reference to the above-mentioned literary sources.

1. Natural selection is the factor responsible for the existence of the individual plasticity of organisms.

2. This plasticity is expressed in the first place by the development of non-reversible morphological adaptive modifications when the environment changes, and secondly by regulatory processes and complex systems of easily reversible physiological changes including reflexes. Due to the regulation of the various changes induced by the environment, development becomes less dependent upon the surrounding conditions; but the seeming 'emancipation of the organism from its environment' is compensated by more perfect and diverse regulatory and functional adaptability.

3. Selection creates and perfects reactions of a complex type that are common among many different species of organisms. This facilitates the rapid and coordinated adaptation of the organism to the new environment. It explains the existence of the so-called 'modification reserve' and often attributes a leading role in evolution to adaptive modifications.

4. Adaptive modifications engender the isolation of consanguineous varieties when interspecific divergence occurs.

5. The selection of partly coincident variations leads to the fixation of non-hereditary differences. This fixation is achieved through the creation of new correlatory mechanisms in the course of selection as well as through the destruction of superfluous ones. Thus adaptability is changed by selection. This denotes the readjustment between the organism

and the environment, the mutual connexion of which was violated when the latter was altered.

6. Coincident selection is the selection of small physiological mutations: they acquire the greatest value in this phenomenon. Such alteration of the basic physiological characters is the result of direct natural selection. Thus the appearance of stable hereditary differences in physiological characters at the earliest stages of divergent evolution finds its explanation. The direct selection of viability through physiological characters in the presence of sharply modified organs leads to the partial fixation of the adaptively modified structures. Therefore such selection happens to be at the same time coincident selection.

7. Adaptive modifications indicate the possible channels of evolutionary transformation, they accelerate and facilitate the latter. Coincident selection perfects non-hereditary adaptations.

8. Selection that stabilizes development, making it less dependent upon the environment, may be considered as one of the forms of coincident selection if adaptive modifications are present. Stabilizing selection is often but not always connected with the increase of the constancy of ecological factors. It increases the significance of internal factors in development. Coincident selection leads *to the fixation of diversity* between populations whereby the adaptability is by no means in all cases decreased. Stabilizing selection on the contrary leads *to the fixation of characters* to a certain degree.

9. The advantages of the surviving forms and not the automatic exclusion of deviations from the normal type are of decisive significance in stabilizing selection.

10. The evolutionary process as a whole is characterized by progressive stabilization of development, particularly in the earlier embryonic stages. Nevertheless this general tendency should not be mistaken for stabilizing selection, the existence of which may be proved only by direct experiments.

11. *Stabilizing selection* lowers the variability and increases general regulatory ability of the organism including the regulation of mutations. Regulation of heterozygous mutations signifies an increased dominance of the 'wild type'. When the dependence of development upon external conditions is increased, the results are just opposite of what has been said above. In some cases of *coincident selection* variability may be altered but it does not necessarily decrease or increase.

12. Coincident (organic) selection explains the parallelisms between hereditary and non-hereditary variability without adhering to the Lamarckian principle of the adequate transmission of acquired characters or to any other orthogenetical conceptions. This fixation can be easily explained by the usual Darwinian factors of evolution, namely variability, heredity, the struggle for life, and natural selection.

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REFERENCES

BALDWIN, J. M. (1896). *Amer. Nat.* **30** (354, 355), 441–51, 536–53.

BALDWIN, J. M. (1902). *Development and Evolution.* 395 pp. London.

BAUR, E. (1922). *Einführung in die experimentelle Vererbungslehre.* 6th ed. 436 pp. Berlin.

BERG, L. S. (1934). *Bull. Acad. Sci. U.R.S.S.*, ser. math. et biol., **5**, 711–32 (in Russian).

BERG, R. L. (1942*a*). *C.R. Acad Sci. U.R.S.S.* **36** (2), 79–83.

BERG, R. L. (1942*b*). *C.R. Acad. Sci. U.R.S.S.* **36** (9), 304–9.

BERG, R. L. (1944). *Bull Acad. Sci. U.R.S.S.*, ser. biol., **5**, 300–7 (in Russian).

BERG, R. L. (1945). *Bull. Acad. Sci. U.R.S.S.*, ser. biol., **3**, 367–75 (in Russian).

DARWIN, CH. (1859). *The Origin of Species by Means of Natural Selection.* London.

DETTO, V. (1904). *Die Theorie der direkten Anpassung und Ihre Bedeutung für das Anpassung- und Deszendenzproblem.* 214 pp. Jena.

DOBZHANSKY, TH. & QUEAL, M. L. (1938). *Genetics*, **23** (2), 239–51.

FILATOV, D. P. (1939). *The Comparative-Morphological Course in the Physiology of Development.* 119 pp. Moscow-Leningrad (in Russian).

FILATOV, D. P. (1941). *J. Gen. Biol.* **2** (1), 3–18 (in Russian).

FISHER, R. A. (1928). *Amer. Nat.* **62** (679), 115–26.

GABRIEL, M. L. (1944). *J. Exp. Zool.* **95** (1), 105–43.

GAUSE, G. F. (1940*a*). *J. Gen. Biol.* **1** (1), 105–20 (in Russian).

GAUSE, G. F. (1940*b*). *J. Zool.* **19** (3), 363–78 (in Russian).

GAUSE, G. F. (1941*a*). *J. Gen. Biol.* **2** (2), 193–209 (in Russian).

GAUSE, G. F. (1941*b*). *Adv. Mod. Biol.* **14** (2), 227–42 (in Russian).

GAUSE, G. F., SMARAGDOVA, N. P. & ALPATOV, W. W. (1942). *Amer. Nat.* **76** (762), 63–74.

GOLDSCHMIDT, R. (1938). *Physiological Genetics.* 375 pp. New York and London.

GULICK, J. T. (1905). Evolution, racial and habitudinal. *Publ. Carneg. Instn*, no. 25. 269 pp. Washington.

HOGBEN, L. T. (1940). *The New Systematics*, pp. 269–86. Oxford.

HUBBS, C. L. (1926). *Amer. Nat.* **60** (666), 57–81.

HUBBS, C. L. (1940). *Amer. Nat.* **74** (752), 198–211.

HUXLEY, J. (1942). *Evolution. The Modern Synthesis.* London.

KALABUCHOV, N. J. (1940*a*). *J. Gen. Biol.* **1** (2), 285–92 (in Russian).

KALABUCHOV, N. J. (1940*b*). *Sci. Bull. Leningr. Univ.* **59**, 80–101 (in Russian).

KAMSHILOV, M. M. (1939). *C.R. Acad. Sci. U.R.S.S.* **23** (4), 361–4.

KAMSHILOV, M. M. (1941). *J. Gen. Biol.* **2** (1), 109–28 (in Russian).

KIRPICHNIKOV, V. S. (1933). *J. Biol.* **2** (6), 609–27 (in Russian).

KIRPICHNIKOV, V. S. (1935). *J. Biol.* **4** (5), 775–801 (in Russian).

KIRPICHNIKOV, V. S. (1940). *J. Gen. Biol.* **1** (1), 121–52 (in Russian).

KIRPICHNIKOV, V. S. (1943). *Bull. Acad. Sci. U.R.S.S.*, ser. biol., **4**, 189–220 (in Russian).

KIRPICHNIKOV, V. S. (1944*a*). *J. Gen. Biol.* **5** (3), 172–92 (in Russian).

KIRPICHNIKOV, V. S. (1944*b*). *Adv. Mod. Biol.* **18** (3), 314–39 (in Russian).

KIRPICHNIKOV, V. S. (1945). *C.R. Acad. Sci. U.R.S.S.* **47** (7), 521–5.

KRUSHINSKY, L. V. (1944). *J. Gen. Biol.* **5** (5), 261–83 (in Russian).

KRUSHINSKY, L. V. (1946). *Bull. Acad. Sci. U.R.S.S.*, ser. biol., **1**, 69–81 (in Russian).

LUKIN, E. J. (1935). *Proc. Kharkov Univ.* **2–3**, 155–70 (in Russian).

LUKIN, E. J. (1936). *Proc. Kharkov Univ.* **6–7**, 199–209 (in Russian).

LUKIN, E. J. (1939). *Adv. Mod. Biol.* **11** (2), 241–66 (in Russian).

LUKIN, E. J. (1940). *Darwinism and the Geographical Conformities in the Changes of Organisms.* 311 pp. Moscow-Leningrad (in Russian).

LUKIN, E. J. (1942). *J. Gen. Biol.* **3** (4), 235–61 (in Russian).

MASHKOVZEFF, A. A. (1936). *Bull. Acad. Sci. U.R.S.S.*, ser. math. et biol., **5**, 945-97 (in Russian).

MASHKOVZEFF, A. A. (1940). *J. Gen. Biol.* **1** (1), 153–76 (in Russian).

MORGAN, LLOYD (1896). *Habit and Instinct.* London and New York.

MULLER, H. J. (1942). *Biol. Symp.* **6**, 71–125.

MURETOV, G. D. (1939). *C.R. Acad. Sci. U.R.S.S.* **24** (5), 481–4.
MURETOV, G. D. (1941). *J. Gen. Biol.* **2** (2), 273–300 (in Russian).
NAUMENKO, V. A. (1941). *C.R. Acad. Sci. U.R.S.S.* **32** (1), 75–8.
OSBORN, H. F. (1896). *Trans. N.Y. Acad. Sci.* pp. 141–8.
OSBORN, H. F. (1897). *Science*, N.S., **6**, 583–7.
PROMPTOV, A. N. (1934*a*). *J. Zool.* **13** (3), 409–36 (in Russian).
PROMPTOV, A. N. (1934*b*). *J. Zool.* **13** (4), 616–28 (in Russian).
PROMPTOV, A. N. (1936). *J. Biol.* **5** (6), 1001–10 (in Russian).
ROUX, W. (1881). *Der Kampf der Theile im Organismus.* 244 pp. Leipzig.
SCHMALHAUSEN, J. J. (1938). *The Organism as a whole in Individual and Historical Development*, 209 pp. (2nd ed. 1942). Moscow-Leningrad (in Russian).
SCHMALHAUSEN, J. J. (1939). *The Channels and Conformities of Evolution.* 229 pp. Moscow-Leningrad (in Russian).
SCHMALHAUSEN, J. J. (1940). *J. Gen. Biol.* **1** (3), 349–70 (in Russian).
SCHMALHAUSEN, J. J. (1941). *J. Gen. Biol.* **2** (3), 307–54 (in Russian).
SCHMALHAUSEN, J. J. (1946). *The Factors of Evolution*, 393 pp. Moscow-Leningrad (in Russian).
SMARAGDOVA, N. P. (1940). *J. Zool.* **19** (2), 211–17 (in Russian).
SMARAGDOVA, N. P. (1941). *J. Gen. Biol.* **2** (1), 73–84 (in Russian).
SMARAGDOVA, N. P. & GAUSE, S. F. (1939). *J. Zool.* **18** (4), 154–62 (in Russian).
SVETOZAROV, E. & STRAICH, G. (1941). *Adv. Mod. Biol.* **14** (1), 1–29 (in Russian).
TACHTADGJAN, A. (1941). *Bull. Armenian Branch Acad. Sci. U.S.S.R.* **2** (7), 57–61 (in Russian).
THELLUNG, A. (1930). *Die Entstehung der Kulturpflanzen.* 91 pp. Freising-München.
THORPE, W. H. (1940). *The New Systematics*, pp. 341–64. Oxford.
TURESSON, G. (1922). *Hereditas, Lund*, **3**, 211.
WADDINGTON, H. H. (1942). *Nature, Lond.*, **150** (3811), 563–5.

A SEX-LINKED AND SEX-LIMITED WHITE-EYED MUTATION OF THE BLOW-FLY (*CALLIPHORA ERYTHROCEPHALA*)

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1. Introduction

White-eyed females of the common blow-fly, *Calliphora erythrocephala*, occurred sporadically in one cage during the breeding of this fly in the laboratory for biological tests in relation to emergency problems during the war. Preliminary breeding experiments indicated that the white-eyed mutation is both sex-linked and sex-limited, as the character appears in the females but is not shown by males. In order to investigate the mutant form more fully, a pure line of the mutant was obtained by isolating a single white-eyed female and inbreeding the progeny. For cross-mating experiments pure-line wild-type cultures were made by catching and isolating wild females, collecting eggs from them and inbreeding the progeny of each female. The wild-type females were caught several miles away from Cambridge in case the mutant gene might be prevalent in flies in the vicinity of the laboratory.

2. Methods

To obtain reliable data for genetical studies of blow-flies it is essential that the food of larvae and the pabulum upon which the females oviposit should be protected from contamination by wandering larvae of different strains or species of flies. To insure the purity of cultures, water barriers must be interposed between the food and any surface upon which may occur wandering larvae, arising from neighbouring cultures or from eggs deposited in the vicinity by stray flies. The technique developed for breeding pure-line cultures has been described in detail elsewhere (Tate, 1947*a*).

The adults are kept in cages about 25 cm. square and are fed on dextrose and meat, preferably fresh horse-flesh. The meat used for feeding flies or larvae is first dipped in boiling water to destroy extraneous larvae or eggs. The mature larvae are allowed to

pupate in sawdust and are kept in covered glass dishes until the adult flies emerge. Cultures are kept at approximately 21° C. At this temperature the approximate length of the various stages is: larval life 6 days, pupal life 14 days, oviposition 7 days after emergence of the females; eggs hatch in 24 hr. Thus the complete life cycle takes about 4 weeks.

3. THE MUTANT EYE

The eyes of newly emerged mutant females are nearly devoid of pigmentation and appear nearly white or tinged with yellow. As the females age there is a gradual increase in the yellow tinge until the eyes assume a golden-yellow colour which may be described as 'apricot colour'. Once this stage is reached there is no further increase in depth of colour. No correlation has been noticed between the mutant eye colour and other characters in the females. Females heterozygous for the mutant eye factor are not distinguishable from wild-type females, and no difference could be seen between the eye colour of wild-type males and of males bearing the eye-mutant factor. The structure of normal and mutant eyes in *C. erythrocephala* and the development of pigment during pupal life have been described in detail elsewhere (Tate, 1947*b*). The mutant eye is normal in structure and contains the full complement of pigment cells, but there is complete suppression of the formation of pigment granules. The yellow colour, which deepens with age, is due to the presence of a diffuse, non-granular pigment in the retinulae.

It will be convenient to symbolize the mutant gene by w, and the normal, wild-type, allelomorph by $+$. Where it is desired merely to indicate the appearance of the flies without specifying their actual genetical constitution for the mutant gene, white-eyed females will be called 'white', and females with normal coloured eyes will be termed 'wild' whether they are homozygous or heterozygous for the wild-type gene; and the males will be called 'wild' whether they carry the mutant gene or not. By analogy with *Drosophila* the white-eyed mutation of *Calliphora* might be labelled 'white-apricot' and symbolized by w^a, but for the sake of simplicity it will be denoted by w.

4. COLOUR OF MALPIGHIAN TUBULES AND TESTES IN WILD-TYPE AND MUTANT FLIES

No difference could be detected in the colour of the Malpighian tubules or of the testes of imagines of the normal and mutant strains. It is known that in *Drosophila* there is a correlation between eye colour and colour of testes (Cochrane, 1938); but in *Calliphora erythrocephala*, as the mutant character is not manifest in the eye of the male bearing the mutant gene, if the same factors govern testis pigmentation as eye pigmentation, the testis colour would not be influenced by the presence of the mutant gene in the male. The Malpighian tubules are very brightly coloured in developing pupae, being yellow proximally and green distally. In the imagines the colours are not so vivid and the green is lost. In no case could constant differences in colour of the Malpighian tubules be detected between males and females of the mutant strain or between normal and mutant females.

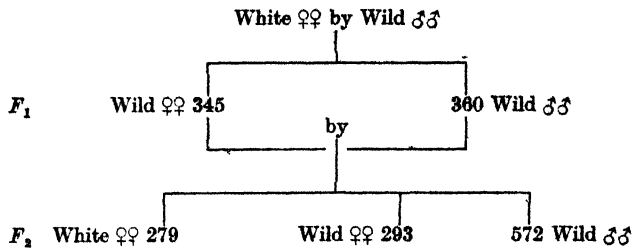
5. RESULTS OF BREEDING EXPERIMENTS

(*a*) *Inheritance of the mutant gene* (w)

When white-eyed females are crossed with pure-line wild-type males the F_1 progeny consists of approximately equal numbers of males and females all with normal eye colour

(Table 1). If the F_1 progeny are inbred, in the F_2 generation both white-eyed and normal females appear in approximately equal numbers and all the males are wild-type (Table 1). Thus, heterozygous females have eyes normal in appearance, and males heterozygous for the mutant gene are also normal in appearance. The occurrence of both normal and white-

Table 1. *Results obtained in an experiment in which white-eyed females were crossed with wild-type males. All the F_1 progeny were wild-type, but white-eyed females segregated out in the F_2 generation*



eyed females in the F_2 progeny shows that the white-eyed female is homozygous for the mutant gene ($=ww$) which is recessive to the wild-type gene ($=+$). As the mutant character is expressed only in the double recessive female it would appear that it is sex-linked and is situated in the X-chromosome. If this is so, it would be expected that the mutant character would appear in males which had inherited the w gene in the X-chromosome derived from their white-eyed mother, as the Y-chromosome in Diptera is usually considered to be nearly inert genetically. The fact that white-eyed males do not occur shows that the mutant character is both sex-linked and sex-limited, and it would appear that it is not expressed in the male because the Y-chromosome bears the dominant allelomorph for the wild-type eye character.

(*b*) *Progeny of mutant females*

The progeny of ww females fertilized by wY males should breed true for white-eyed females and wild-type eyed males which bear the w gene in their X-chromosome; but, if they are fertilized by wild-type males ($+Y$) all the F_1 progeny should be wild-type in both males and females, and white-eyed females should appear only in the F_2 generation. Thus, if white-eyed females appear in the F_1 progeny of a white-eyed mother, she must have been fertilized by a male bearing the mutant gene, and all her sons will inherit this gene from her. The results of breeding from such an isolated white-eyed female are shown in Table 2. White-eyed females and wild-type males appeared in the F_1 generation and thereafter, when inbred, the line bred true for eleven successive generations.

The progeny from another single white-eyed female, which presumably had been fertilized by a male bearing the normal allelomorph of the mutant gene in its X-chromosome, is shown in Table 3. In the F_1 generation both males and females had wild-type eyes, but when inbred they gave F_2 progeny consisting of nearly equal numbers of both white-eyed and wild-type females and all wild-type males. Random fertilization was allowed to occur in the F_2 progeny, and one of each type of female was isolated and the F_3 progeny raised. The white-eyed female gave only white-eyed females and wild-type males, and these when inbred continued to breed true for six further generations. The wild-type female, which should be heterozygous for the mutant gene, gave nearly equal

numbers of white-eyed and wild-type females and only wild-type males, as would be expected if she had been fertilized by a male bearing the mutant gene. Another true-

Table 2. *The progeny of an isolated white-eyed female for the F_1 to F_{11} generations. The parental female had apparently been fertilized by a male bearing the mutant gene*

Isolated white ♀

F_1	White ♀♀ 92	87 Wild ♂♂
	by	
F_2	White ♀♀ 264	368 Wild ♂♂
F_3	White ♀♀ 231	342 Wild ♂♂
F_4	White ♀♀ 258	398 Wild ♂♂
F_5	White ♀♀ 161	311 Wild ♂♂
F_6	White ♀♀ 230	252 Wild ♂♂
F_7	White ♀♀ 196	287 Wild ♂♂
F_8	White ♀♀ 133	178 Wild ♂♂
F_9	White ♀♀ 247	382 Wild ♂♂
F_{10}	White ♀♀ 244	362 Wild ♂♂
F_{11}	White ♀♀ 163	255 Wild ♂♂

Table 3. *The results obtained by breeding the F_1–F_{10} progeny from an isolated white-eyed female which had been fertilized by a wild-type male. The F_3 generation was raised from a single white-eyed female and a single wild-type female (heterozygous) of the F_2 generation*

White ♀

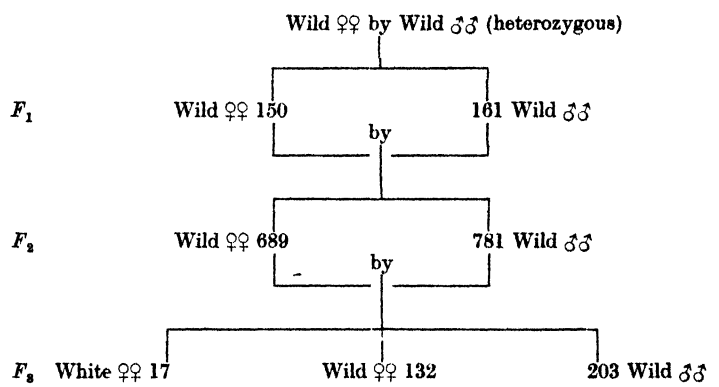
F_1	Wild ♀♀ 17		11 Wild ♂♂		
	by				
F_2	White ♀♀ 214	Wild ♀♀ 248		602 Wild ♂♂	
	by				
	(Isolated ♀)	by			
		(Isolated ♀)			
F_3	White ♀♀ 17	31 Wild ♂♂	White ♀♀ 40	Wild ♀♀ 49	73 Wild ♂♂
	by		by		
F_4	White ♀♀ 63	115 Wild ♂♂	White ♀♀ 66		54 Wild ♂♂
F_5	White ♀♀ 19	37 Wild ♂♂	White ♀♀ 64		64 Wild ♂♂
F_6	White ♀♀ 40	53 Wild ♂♂	White ♀♀ 220		290 Wild ♂♂
F_7	White ♀♀ 58	73 Wild ♂♂	White ♀♀ 108		140 Wild ♂♂
F_8	White ♀♀ 60	81 Wild ♂♂	White ♀♀ 95		86 Wild ♂♂
F_9	White ♀♀ 85	81 Wild ♂♂	White ♀♀ 83		121 Wild ♂♂
F_{10}	White ♀♀ 22	43 Wild ♂♂	White ♀♀ 37		126 Wild ♂♂

breeding white-eyed strain was extracted in the F_4 generation from the F_3 progeny of this female.

(c) *The result of crossing wild-type females with males heterozygous for the white-eyed mutant gene*

If, as has been postulated above, males bred from white-eyed females bear the mutant gene in their X-chromosome, when they are crossed with wild-type females they should give, in the F_1 generation, heterozygous females with wild-type eyes and wild-type males. If these are inbred, in the F_2 generation all the females should have wild-type eyes, but they should be of two kinds genetically: (*a*) homozygous wild-type, and (*b*) heterozygous wild-type; and the males should also consist of two genetical types in equal numbers, that is (*a*) males with the mutant gene in the X-chromosome, and (*b*) males not bearing the mutant gene. When the F_2 progeny are inbred segregation should occur in the F_3 generation, and both white-eyed and normal females should appear in the proportion of 1:7 and, as usual, all the males should be wild-type. The results of such a cross are shown in Table 4, and it can be seen that the results agree well with those expected. In another

Table 4. *Results obtained by crossing wild-type females with males bearing the mutant gene (heterozygous). All the progeny have wild-type eyes in the F_1 and F_2 generations, but white-eyed females segregate out in the F_3 generation*



experiment (Table 5) it was found that if the F_3 white-eyed females are inbred with the F_3 males, white-eyed and wild-type females appear in the F_4 generation. Inbreeding these white-eyed females and wild-type males, which all bear the mutant gene in their X-chromosome (as they have white-eyed mothers), gives in the F_5 generation only white-eyed females and wild-type males; and these, when inbred, breed true. The results of this experiment are shown in Table 5, and, although the number of adults bred is not large enough to give reliable proportional figures, they are in general agreement with the results postulated.

The crossing of heterozygous females with heterozygous males (that is, males from a white-eyed mother) should give F_1 progeny consisting of white-eyed and wild-type females and wild-type males in the proportion 1:1:2. Table 6 gives the result of a breeding experiment in which this cross was made and the figures obtained for the F_1 generation, 206 white-eyed females, 217 wild-type females and 450 wild-type males, are very near to the predicted proportions.

Table 5. *Results obtained in an experiment in which a single wild-type female was mated to a single wild-type male carrying the mutant gene. White-eyed females segregated out in the F_3 generation and a true-breeding white-eyed line was obtained in the F_5 generation by breeding from them*

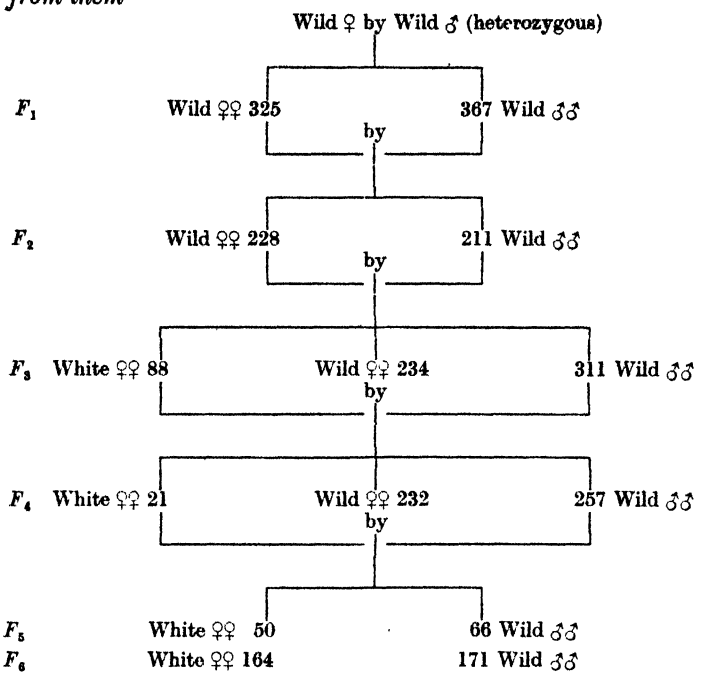
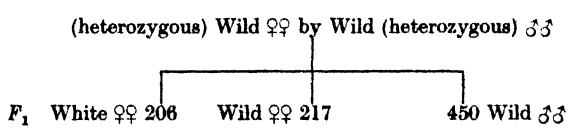


Table 6. *Results obtained when wild-type females heterozygous for the mutant gene were crossed with wild-type heterozygous males. In the F_1 generation nearly equal numbers of white-eyed and wild-type females were obtained; and, as usual, all the males were wild-type*



(*d*) *Interpretation of the results of breeding experiments*

Many other breeding experiments gave similar results and records of 52,245 descendants of a single white-eyed female have been collected. In these experiments there were 16,952 white-eyed females, 7185 wild-type heterozygous females and 28,108 presumably heterozygous wild-type males, but a male with white eyes was never observed. This is strong evidence for the absence of crossing-over in the male *C. erythrocephala*, at least in the active part of the sex chromosomes. Some anomalous results were obtained which will be considered later, but all the results indicate that the mutant gene is sex-linked and appears only in the double recessive condition. It is also sex-limited, as it does not appear in the heterozygous male. The general rule in Diptera is that the sex chromosomes of the female are *XX* and of the male *XY*, and it seems clear that the mutant gene is located in the *X*-chromosome. Its failure to be expressed in the male when it is present in the single *X*-chromosome may be due to the occurrence of a dosage effect, a double

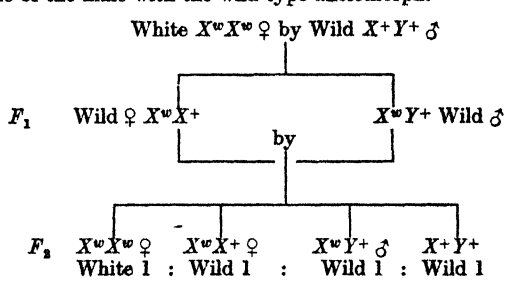
dose of the mutant gene being necessary for its expression, but it may also be postulated that a normal allelomorph is present in the Y-chromosome. The fact that heterozygous females are not distinguishable from the homozygous wild-type females and that, similarly, heterozygous males do not differ in eye colour from wild-type males suggests that it is not a dosage effect, but that a normal allelomorph of the mutant gene is present in the Y-chromosome in the male.

If the X-chromosome bearing the mutant gene is represented by X^w, the X-chromosome with the normal allelomorph by X^+ and the Y-chromosome of the male with the normal allelomorph by Y^+ the mode of inheritance of the mutant gene can be shown diagrammatically as in Tables 7–9.

The result to be expected from crossing a white-eyed mutant female with a wild-type male is shown in Table 7. In the F_1 generation all the progeny will be wild-type but, genetically, both males and females will be heterozygous for the mutant gene. If they are inbred in the F_2 generation four genetical types will arise: white-eyed females; wild-type

Table 7. *Scheme showing the mode of inheritance of the mutant character in the progeny of a white-eyed female mated to a wild-type male if the mutant gene is located in the X-chromosome and has a wild-type allelomorph in the Y-chromosome of the male. Compare with Tables* 1 *and* 3

X^w = an X-chromosome bearing the mutant gene.
X^+ = an X-chromosome bearing the wild-type allelomorph.
Y^+ = the Y-chromosome of the male with the wild-type allelomorph.



females, heterozygous for the mutant gene; wild-type males bearing the mutant gene; and wild-type males without the mutant gene, that is, homozygous for the dominant wild-type gene. These types will be distinguishable morphologically into white-eyed females, wild-type females, and wild-type males in the proportions 1:1:2. If this scheme is compared with the results of breeding experiments shown in Tables 1 and 3, it is evident that there is close agreement.

The result of crossing a wild-type female with a male bearing the mutant gene in its X-chromosome is shown in Table 8. In the F_1 generation all the progeny have wild-type eyes, but the females are heterozygous and the males are homozygous for the dominant allelomorph. In the F_2 generation all the progeny have wild-type eyes, but there are four genetical types: heterozygous females, homozygous females, heterozygous males and homozygous males. If all these types are inbred in the F_3 generation white-eyed females will appear among the five genetical types present. The five types are: double recessive females (white-eyed), wild-type females heterozygous for the mutant gene; wild-type females homozygous for the dominant wild-type gene; and males with and without the mutant gene in the X-chromosome. These five genetical types should appear in the

proportions 1:4:3:2:6. Morphologically they may be distinguished as white-eyed females, wild-type females, and wild-type males in the proportions 1:7:8. Again the results of breeding experiments agree with those predicted in the above scheme for this cross (Table 4).

If white-eyed females are crossed with males heterozygous for the mutant gene (X^wY^+) they will breed true for successive generations, always giving white-eyed females and wild-type males (Table 9). In fact, breeding experiments have always given this result (Tables 2, 3, 5).

Table 8. *Scheme showing the mode of inheritance of the mutant character in the progeny of a wild-type female mated to a male carrying the mutant gene in its X-chromosome. Segregation of the white-eyed females should occur in the F_3 generation and there should be, in this generation,* 1 *white-eyed female to* 7 *wild-type females to* 8 *wild-type males. Compare this scheme with the results of the breeding experiments recorded in Table* 4

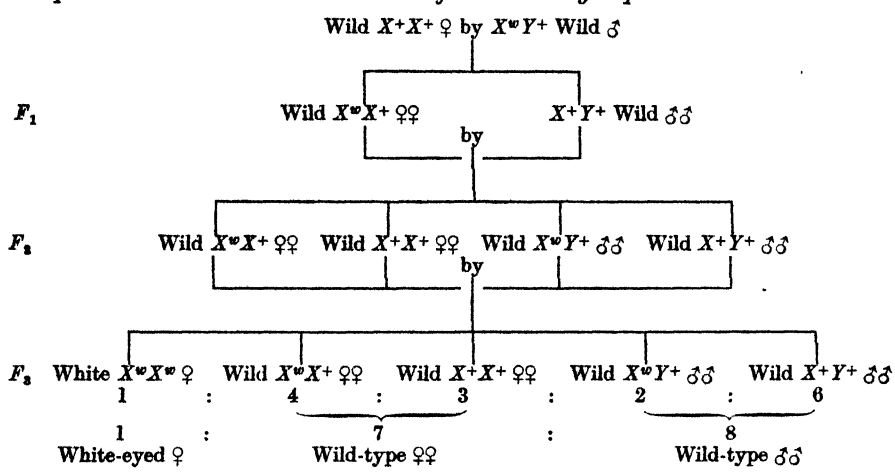
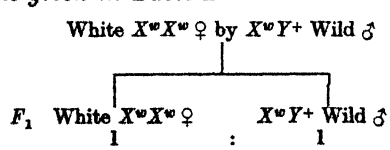


Table 9. *Scheme showing the mode of inheritance in the progeny of a white-eyed female mated to a wild-type male carrying the mutant gene. The stock will breed true for successive generations giving white-eyed females and wild-type, heterozygous, males. Compare with the experimental results given in Table* 2



Thus the evidence is strongly in favour of the view that the mutant gene is recessive to the wild-type gene and is located in the X-chromosome. In the male there is a wild-type allelomorph in the Y-chromosome which is dominant over the mutant gene in the X-chromosome, and hence the mutation is sex-limited and is visible only in the double recessive female. At present there is no evidence for the occurrence of crossing-over between the X- and Y-chromosomes in the male of *C. erythrocephala*.

In discussing the genetical analysis of a sex-limited character in *Drosophila melanogaster*, Gordon & Gordon (1939) have pointed out that three types of genetical difference might be expected to give rise to sex-limited characters: (*a*) when genes whose effects are

incompletely dominant and additive are located in the *X*-chromosome, the so-called 'dosage' effect; (*b*) the specific effect of *Y*-borne genes; and (*c*) when the sex-limited character depends directly or indirectly upon the genetic difference which is also responsible for the primary distinction between the sexes, or is determined by a gene balance of the same general type. If the first type of genetical difference were responsible for the sex-limitation of the mutant character in *Calliphora erythrocephala* some difference in appearance would be expected between homozygous and heterozygous wild-type females, but no such difference has been observed. If the sex-limitation of the character in *C. erythrocephala* were due to the third type of genetical difference, then there should be grades of expression of the mutant character from minimal to maximal as in the case of 'Brown palp' in *Drosophila melanogaster* described by Gordon & Gordon (1939). The specific effect of a *Y*-borne gene, in this case a normal allelomorph of the mutant gene, remains as the most probable explanation of the sex-limitation of the mutant character described for *Calliphora erythrocephala*.

6. Anomalous females in white-eyed cultures

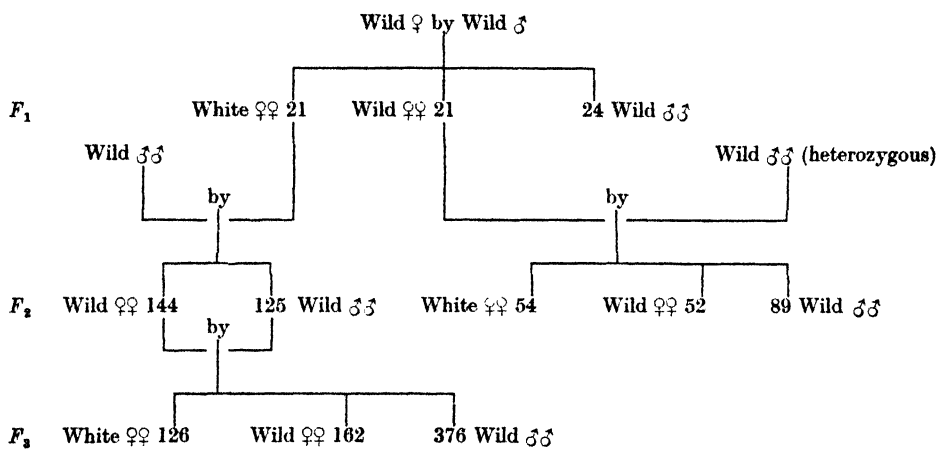
In some pure-line white-eyed cultures after many generations of breeding true, abnormal females with wild-type eyes occurred. It is uncertain how these females originated, and they may have arisen from some genetical alteration in the chromosome bearing the mutant gene. The possibility of contamination cannot be excluded owing to the technical difficulties of maintaining pure-line cultures of *Calliphora*, and it is considered to be the most likely explanation until there is more evidence for one of the other views. Crossing-over in the male between the active portions of the *X*- and *Y*-chromosomes would account for the occurrence of the wild-type females in the pure-line white-eyed cultures; but, in that case, white-eyed males should also arise as a result of the crossing-over, and the absence of any such males renders this explanation unlikely. It is possible that non-disjunction of *X*- and *Y*-chromosomes might result in the appearance of wild-type females, but there is no evidence for this view.

One of the anomalous females was paired with a wild-type (heterozygous) male bred from the same batch of larvae, and the results of this pairing are shown in Table 10.

In the F_1 generation 21 white-eyed females, 21 wild-type females and 24 wild-type males were obtained. There appears to be a deficiency of males in this generation, but the deficiency is not significant when tested statistically. Both types of females were tested for their genetical constitution by being outcrossed with males of known genetical constitution as regards the mutant gene. The white-eyed females were paired with wild-type males (homozygous for the wild-type dominant gene), and in the following generations segregation took place in the manner to be expected from such a cross. The F_1 wild-type (presumably heterozygous) females from the abnormal female were paired with a wild-type male heterozygous for the white-eyed gene, and in the next generation they gave white-eyed females and wild-type males in the ratios 1:1:2, as would be expected from the cross between heterozygous females and heterozygous males. No abnormal types were observed in any of the progeny in these experiments. It would appear that the original abnormal female was heterozygous for the mutant character, and there is no evidence to support any chromosomal change such as crossing-over or non-disjunction, and the explanation for the occurrence of such females must be sought, in all probability, either in contamination or reverse mutation. The critical test for non-

disjunction would have been to cross the 24 F_1 males with white-eyed females, but it was not possible to carry out this test at the time and abnormal females have not been found since. It has been shown by Johnston & Winchester (1934) that in *Drosophila melanogaster* reverse mutations occur in X-rayed males, but that the frequency of reverse mutations is only 1:12 compared with the frequency of direct mutations. Of the genes studied 'Forked' reverted most frequently, the rate being about 1:12,000 flies.

Table 10. *The results of breeding from an anomalous wild-type female that appeared in a pure-line white-eyed culture. The two kinds of females in the F_1 progeny, white-eyed and wild-type, were out-crossed with pure-line wild-type and heterozygous males to test their genetical composition with respect to the mutant gene. The F_2 and F_3 progeny show that the mutant gene is inherited in the normal manner. The results indicate that the anomalous female was heterozygous for the mutant gene and that no genetical change had taken place in the chromosomes bearing the mutant gene*



If reverse mutations are the explanation of the anomalous females in *Calliphora erythrocephala* cultures it would seem that the rate is very high, as it has occurred with a frequency of about 1:10,000 flies which is greater than the highest rate in X-rayed *Drosophila*. On the other hand, in the 26,000 flies raised from various wild-type lines no spontaneous occurrence of the mutant eye has been seen. This would mean that the reverse mutation is more frequent than direct mutation in *Calliphora erythrocephala*. It is therefore unlikely that reverse mutation has taken place so frequently in the cultures. It requires much further work, with larger numbers of flies bred under conditions precluding contamination of the cultures, to decide whether or not reverse mutation does occur.

7. THE CHROMOSOMES OF *CALLIPHORA ERYTHROCEPHALA*

(a) *Meiosis in gonads of larvae and pupae*

In the testes of newly emerged males various stages of meiotic division may be found, but mostly the testes are packed with more or less developed spermatozoa. Gonads from pupae are the best material for studying meiosis, and it was found that if pupae are selected as soon as the puparium has hardened, but not darkened, and then incubated at 24° C., division stages could be obtained with great regularity by dissecting the gonads from pupae of appropriate age and making aceto-carmine squash preparations of them.

Lowne (1893–5) states that the earliest stage at which he could identify the gonads with certainty, in section, was the first day of pupal life, and that he succeeded in dissecting out both ovaries and testes on the third day of pupal life. In the present work it was found that both ovaries and testes could be dissected out from third instar larvae, even before they had emptied the gut prior to pupation. The gonads at this stage are embedded ventrolaterally in the fat-body slightly anterior to the level where the third ventral branch, counting from the posterior spiracle, arises from the lateral tracheal trunk. The ovaries are situated more ventral and anterior to the testes. In gonads removed from larvae no meiotic stages were seen.

In testes from pupae incubated at 24° C. only diploid division stages are present during the first 72 hr. when spermatogonia are actively dividing. At 96 hr. (4 days) meiotic divisions may be found, and numerous first spermatocyte divisions are present. At 126 hr. spermatogonia, first spermatocyte and second spermatocyte divisions are all present and, already, developing spermatozoa may be recognized. Apparently mature spermatozoa are formed by 150 hr. (6 days) and thereafter, for the rest of pupal life, which is 240 hr. at 24° C.; all stages of development from spermatogonial divisions to the fully formed spermatozoa may be found in preparations of the testes.

The best material for observing all stages of meiosis is testes from pupae after 4–6 days' incubation at 24° C.

(*b*) *The sex chromosomes of* Calliphora erythrocephala

Metz (1916) depicted the chromosome complex of *C. erythrocephala* as consisting of six pairs, of which five are large and one, the sixth, is formed of a pair of very small rod-shaped chromosomes. Stevens (1908) described a very similar complex for *C. vomitoria* and interpreted the small pair as being the sex chromosomes, which are heterologous in the male, one larger and one small, and homologous in the female, consisting of a pair of equal-sized rods. More recently Keuneke (1924) studied the spermatogenesis of *C. erythrocephala* and described the chromosome complex as consisting of five equal pairs and a sixth pair of unequal heterochromosomes, one of which has the form of a short, thick rod and the other that of a thin rod or sphere. This author did not decide which of the heterochromosomes was the *X* and which the *Y*. Naville (1932) considers the larger of the heterochromosomes, the rod-shaped one, to be the *X*-chromosome, and the smaller, spherical member of the pair to be the *Y*-chromosome.

In the present work on the eye mutant many gonads of developing larvae, pupae and male imagines were examined by the aceto-carmine 'squash' technique (Darlington & La Cour, 1942), but, although the chromosome complex was found to consist of six pairs as described by previous authors, no definite and constant morphological difference could be detected in such preparations in the pair of small chromosomes in the male sex. In some meiotic divisions one of the pair might appear rod-shaped and the other round, but such differences were not constant and could be interpreted as arising from one member of the pair being seen in side view and the other end-on. The occurrence of chiasmata between this pair of chromosomes, whereas chiasmata were not seen in any of the five other pairs of chromosomes, indicates that the small sixth pair are in fact the sex chromosomes and that the five large pairs are the autosomes.

Examination of the giant salivary gland chromosomes of larvae of *C. erythrocephala* by the aceto-carmine 'squash' technique was not helpful in recognizing the individual

chromosomes because the large amount of heterochromatin present made it impossible to obtain clear and well-separated preparations of the chromosome complex.

According to Naville (1932) there is a fundamental difference in the gametogenesis of the male and female cells in *C. erythrocephala*. Only in the female are the characteristic pre-meiotic phases developed; and in the male the reduction follows directly on the appearance of the prochromosomes without the intervention of stages of leptotene, zyotene, pachytene and diplotene.

The failure in the present work to find any white-eyed males among the 28,000 males bred from white-eyed strains strongly suggests that crossing-over does not occur in the male of *C. erythrocephala* between the centromere and the *w* locus, or that it is a very rare occurrence.

The behaviour of the white-eye mutant gene in *C. erythrocephala* may be compared with the mutant 'bobbed' in *Drosophila melanogaster* (Mohr, 1923). This is a sex-linked mutant, limited almost entirely to the female sex. It is recessive and is situated far to the right on the *X*-chromosome, and there appears to be a normal allelomorph in the *Y*-chromosome. Muller & Painter (1932) have pointed out that 'bobbed' is the only 'visible' mutation located in the right-hand third of the *X*-chromosome; and, according to Philip (1935), this third of the chromosome, except for the factor *bb*, is inert and consists of heterochromatin. Schubel (1934) has shown, however, that a sex-linked and female-limited lethal in *D. melanogaster* is located near the locus of 'bobbed' and has a normal allelomorph in the *Y*-chromosome. As the *Y*-chromosome carries the normal allelomorph for 'bobbed', but is otherwise practically inert except for the K_1 and K_2 fertility complexes (Philip, 1935), it seems that the right-hand third of the *X*-chromosome is homologous with the *Y*-chromosome in the region where the mutant genes occur. Although Muller & Painter (1932) have stated that little or no crossing-over occurs between the right-hand half of the *X*-chromosome and the *Y*-chromosome, Philip (1935) has shown that crossing-over of 'bobbed' between the *X*- and *Y*-chromosome in *D. melanogaster* did occur with a frequency of about 1:3000 of her experiments. Darlington (1934) considers that the absence of crossing-over in the male of *D. pseudo-obscura* is due to the fact that, although pairing between the *X*- and *Y*-chromosomes is by reciprocal chiasmata, the regions involved are inert and hence no visible crossing-over results.

In the case of *Calliphora erythrocephala*, by analogy with *Drosophila*, it would seem that as the normal allelomorph of the white-eyed factor occurs in the *Y*-chromosome the mutant gene must be located in the homologous portion of the *X*-chromosome. Hence, if pairing of the *X*- and *Y*-chromosomes in the male is chiasmatype, crossing-over involving this region should occur and result in the appearance of white-eyed males; but the results of breeding experiments provide no evidence for the occurrence of crossing-over. Unfortunately, the minute size of the heterochromosomes in *Calliphora* renders microscopical observation of the pairing of these chromosomes during meiosis very difficult, and the nature of the various types of chiasma formation observed has not been determined.

8. Discussion

In *Drosophila* many eye mutants are sex-linked, but the inheritance of the mutant genes is such that the character is expressed in the male bearing the mutant gene in the *X*-chromosome, and hence a normal allelomorph cannot be present in the *Y*-chromosome.

White-eyed mutants have been recorded for other Diptera by Turner (1923) in *Psychoda alternata*, by Mackerras (1933) in *Lucilia cuprina* Wed., and by Dichler (1943) in *Phormia regina* Meigen; but in all these cases the mutant genes, which behave as Mendelian recessives, appear to be located in one of the autosomes and the mutant character is not sex-linked. Recently, Gilchrist & Haldane (1947) have described a recessive white-eyed mutant in the mosquito *Culex molestus* which is partially linked with sex, but in this insect there are no sex chromosomes, and maleness appears to be due to a single dominant gene in the same chromosome as the gene for white eye.

Thus, genes controlling eye pigmentation are located in the *X*-chromosome in *Drosophila* and *Calliphora*, and it is strange that in flies so closely related to *Calliphora* as *Lucilia* and *Phormia* such genes should be located in autosomes. Dichler (1943) has suggested that if the white-eyed mutants in *Phormia*, *Lucilia* and *Drosophila* are homologous, the fact that the mutant genes are located in the *X*-chromosome in *Drosophila* and in one autosome in *Phormia* and *Lucilia* indicates a translocation between the *X*-chromosome and one of the autosomes or a transfer of the sex-determining factors from chromosome to chromosome in the phylogeny. It would be most interesting if a detailed study of the chromosome complex of normal and mutant forms of *Phormia* and *Lucilia* could be made and results compared with the condition in *Calliphora erythrocephala*, especially as regards the relationship of the *X*- and *Y*-chromosomes in these flies.

The special interest of the white-eyed mutant of *C. erythrocephala* is the fact that there appears to be a wild-type allelomorph present in the *Y*-chromosome so that it is both sex-linked, since the mutant gene appears to be located in the *X*-chromosome, and sex-limited as it cannot be expressed in the male because the normal allelomorph in the *Y*-chromosome is always dominant over the recessive when it is borne by the *X*-chromosome.

The existence of normal allelomorphs in the *Y*-chromosome is a very rare phenomenon and only a few cases have been described in the literature. In *Drosophila* the cases of 'bobbed' (Mohr, 1923) and the lethal described by Schubel (1934) clearly resemble the state of affairs in *Calliphora erythrocephala* described above. In animals genes, other than sex-determining genes, located in the *Y*-chromosome have been demonstrated in the fish *Lebistes reticulatus* by Winge (1922, 1923, 1927), and in other Cyprinodonts by Aida (1921) and Gordon (1947); in the beetle *Phytodecta variabilis* by de Zulueta (1925); and Haldane (1932, 1936) has produced evidence that in man the *Y*-chromosome contains a number of genes which are associated with certain pathological conditions. In plants Knapp (1938) has described a *Y*-linked lethal induced by ultra-violet radiation in the liverwort *Sphaerocarpus Donnellii*; and Löve (1943) has reported an asynaptic gene in *Rumex acetosa* which is located in one of the two *Y*-chromosomes. According to Winge (1931), in *Melandrium*, in addition to three autosomal inhibitors of 'variegated', the *Y*-chromosome also contains a 'variegated' inhibitor so that variegated plants are always female. In *Calliphora* the fact that the appearance of the white-eyed character is limited to females might be explained on similar lines by postulating the presence of an inhibitor of 'white-eye' in the *Y*-chromosome, but at present there is no evidence in support of this view against that of the presence of a normal allelomorph of the mutant gene in the *Y*-chromosome. The latter interpretation is more in agreement with the accepted explanations for the behaviour of 'bobbed' and Schubel's lethal in *Drosophila*.

In the cases in which genes in the *Y*-chromosome have been demonstrated in insects or

other animals, it has been found that crossing-over between the X- and Y-chromosomes does occur, although with a low frequency rate, and the apparent absence of crossing-over in the experiments on the white-eyed mutant of *Calliphora erythrocephala* is difficult to explain, and it is a point which merits further and more detailed investigation.

It should be noted that *Drosophila* in some respects is not typical of Diptera in general. Morgan, Bridges & Sturtevant (1925) pointed out that the type of chromosome group in *Drosophila* is different from the type almost universal in other members of the cyclorrhaphous Diptera. In these Diptera the general rule is six pairs of chromosomes of which the sixth pair is the smallest and consists of rod-shaped or nearly spherical chromosomes, and in all cases where the X-chromosome has been identified it is a member of the smallest pair. Thus, *Calliphora* has the type of chromosome group usual in Diptera, whereas that of *Drosophila* differs from the usual arrangement in that the X- and Y-chromosomes are not the smallest pair of the complex.

Drosophila also differs from many other Diptera in that the males emerge from puparia later than the females, a fact which has been used by Bridges (1922) in support of his views on the influence of opposing sets of genes in determining the sex of the individual. Bridges maintained that the presence of two X-chromosomes in the female *Drosophila* increases the vigour of such individuals and causes more rapid development than in the male in which only one X-chromosome is present. This state of affairs is not general in other Diptera. In *Calliphora* the males develop more quickly than females, and usually the first flies to emerge from a batch of puparia of equal age are males. Mostly males emerge during the first 12 hr. from puparia of *C. erythrocephala*, and the flies which emerge later are mostly females. The same applies also to *Lucilia* and to mosquitoes, at least to the species *Culex pipiens* and *Aëdes ægypti*.

From the results of the breeding experiments described in this paper it is evident that the eye mutant of *Calliphora erythrocephala* is an example of a very rare genetical phenomenon, the presence of a normal allelomorph in the Y-chromosome. The presence of the mutant gene is expressed in so obvious a manner that genetical analysis of crossing experiments is rendered easy. *C. erythrocephala*, once the technical difficulties of breeding pure-line cultures were surmounted, has proved to be a most useful species for genetical study, and the mutant form should prove a valuable addition to the comparatively few mutations available for laboratory work on the relationship of the active and inert fractions of the sex chromosomes.

9. Summary

1. A white-eyed mutant of the common blow-fly (*Calliphora erythrocephala*) has been studied.

2. The eyes of mutant flies are nearly colourless at emergence, but darken with age to an apricot tinge. There is no correlation between eye colour and colour of Malpighian tubules or testes in the mutant flies.

3. The mutant character behaves as a Mendelian recessive and appears only in the double recessive female.

4. The mutant character is both sex-linked and sex-limited, as it is never manifest in the male although the male can transmit the character to its offspring.

5. The results of breeding experiments are interpreted as proving that the mutant gene is located in the X-chromosome; and that there is a wild-type allelomorph present

in the *Y*-chromosome of the male. Thus, the mutation behaves in the same manner as does the character 'bobbed' in *Drosophila*.

6. No evidence was found for the occurrence of crossing-over between homologous parts of the *X*- and *Y*-chromosomes in male blow-flies.

I wish to express my thanks to Prof. D. Keilin for much stimulating advice and criticism, and to Dr D. G. Catcheside, Department of Genetics, Cambridge, for his valuable advice during the work and in revising the manuscript.

REFERENCES

AIDA, T. (1921). On the inheritance of colour in a fresh-water fish, *Aplocheilus latipes* Temmick and Schlegel, with special reference to sex-linked inheritance. *Genetics*, **6**, 554–73.

BRIDGES, C. B. (1922). The origin of variations in sexual and sex-limited characters. *Amer. Nat.* **56**, 51–64.

COCHRANE, F. (1938). Genetic and developmental relationships of testes and eye colour in *Drosophila pseudo-obscura*. *J. Genet.* **36**, 11–16.

DARLINGTON, C. D. (1934). Anomalous chromosome pairing in the male *Drosophila pseudo-obscura*. *Genetics*, **19**, 95–118.

DARLINGTON, C. D. & LA COUR, L. F. (1942). *The Handling of Chromosomes*. London: George Allen and Unwin.

DICHLER, H. (1943). White-eyed mutation in *Phormia regina*. *Amer. Nat.* **77**, 287–8.

GILCHRIST, B. M. & HALDANE, J. B. S. (1947). Sex linkage and sex determination in a mosquito, *Culex molestus*. *Hereditas, Lund*, **33**, 175–90.

GORDON, C. & GORDON, F. (1939). The genetical analysis of a sex-limited character in *Drosophila melanogaster* and its bearing on the evolution of secondary sexual characteristics. *Proc. Roy. Soc.* B, **127**, 487–510.

GORDON, M. (1947). Genetics of *Platypoecilus maculatus*. IV. The sex determining mechanism in two wild populations of the Mexican platyfish. *Genetics*, **32**, 8–17.

HALDANE, J. B. S. (1932). Genetical evidence for a cytological abnormality in man. *J. Genet.* **26**, 341–4.

HALDANE, J. B. S. (1936). A search for incomplete sex linkage in man. *Ann. Eugen., Lond.*, **7**, 28–57.

JOHNSTON, O. & WINCHESTER, A. M. (1934). Studies on reverse mutations in *Drosophila melanogaster*. *Amer. Nat.* **68**, 351–8.

KEUNEKE, W. (1924). Über die spermatogenese einiger Dipteren. *Z. Zell.- u. Gewebelehre*, **1**, 357–412.

KNAPP, E. (1938). Mutationauslösung durch ultraviolettes Licht bei dem Lebermoos *Sphaerocarpus Donnellii*. Aust. *Z. indukt. Abstamm.- u. VererbLehre*, **74**, 54–69.

LÖVE, A. (1943). A *Y*-linked inheritance of asynapsis in *Rumex acetosa*. *Nature, Lond.*, **152**, 358–9.

LOWNE, B. T. (1893–5). *The Anatomy, Physiology, Morphology and Development of the Blow-fly* (Calliphora erythrocephala). London: R. H. Porter.

MACKERRAS, M. J. (1933). Note on the occurrence of a white-eyed mutant race of *Lucilia cuprina* Wild. *Aust. J. Exp. Biol. Med. Sci.* **11**, 45–7.

METZ, C. W. (1916). Chromosome studies on the Diptera. II. The paired association of chromosomes in the Diptera and its significance. *J. Exp. Zool.* **21**, 213–62.

MOHR, O. L. (1923). A genetic and cytological analysis of a section deficiency involving four units of the *X*-chromosome in *Drosophila melanogaster*. *Z. indukt. Abstamm.- u. VererbLehre*, **32**, 108–16.

MORGAN, T. H., BRIDGES, C. B. & STURTEVANT, A. H. (1925). The genetics of *Drosophila*. *Bibliogr. genet.* **2**, 3–262.

MULLER, H. J. & PAINTER, T. S. (1932). The differentiation of the sex chromosomes of *Drosophila* into genetically active and inert regions. *Z. indukt. Abstamm.- u. VererbLehre*, **62**, 316–65.

NAVILLE, A. (1932). Les bases cytologiques de la théorie du 'crossing-over'. Étude sur la spermatogénèse et l'ovogénèse des Calliphorinae. *Z. Zellforsch.* **16**, 440–70.

PHILIP, U. (1935). Crossing-over between *X*- and *Y*-chromosomes in *Drosophila melanogaster*. *J. Genet.* **31**, 340–52.

SCHUBEL, F. (1934). A new sex-linked, female-limited lethal of *Drosophila melanogaster*. *Amer. Nat.* **68**, 279–82.

STEVENS, N. M. (1908). A study of the germ cells of certain Diptera, with reference to the heterochromosomes and the phenomenon of synapsis. *J. Exp. Zool.* **5**, 359–74.

TATE, P. (1947*a*). The technique for breeding pure-line cultures of the blow-fly (*Calliphora erythrocephala*). *Parasitology* (in the Press).

TATE, P. (1947*b*). The structure of normal and mutant eyes in the blow-fly (*Calliphora erythrocephala*) and the development of eye-pigment during pupation. *J. Genet.* (in the Press).

TURNER, C. L. (1923). The Psychodidae (moth-like flies) as subjects for studies in breeding and heredity. *Amer. Nat.* **57**, 545–58.

WADDINGTON, C. H. (1939). *An Introduction to Modern Genetics.* London: George Allen and Unwin.

WINGE, Ö. (1922). One-sided masculine and sex-linked inheritance in *Lebistes reticulatus*. *J. Genet.* **12**, 145–62.

WINGE, Ö. (1923). Crossing-over between the *X*- and the *Y*-chromosome in *Lebistes*. *J. Genet.* **13**, 201–17.

WINGE, Ö. (1927). The location of eighteen genes in *Lebistes reticulatus*. *J. Genet.* **28**, 1–43.

WINGE, Ö. (1931). *X*- and *Y*-linked inheritance in *Melandrium*. *Hereditas, Lund*, **15**, 127–65.

ZULUETA, A. DE (1925). La herencia ligada al sexo en el coleóptero *Phytodecta variabilis* (Ol.). *Eos, Madr.*, **1**, 203–29.

THE EFFECT OF COLD UPON THE DEVELOPMENT OF PIGMENT IN A WHITE-EYED MUTANT FORM OF THE BLOW-FLY (*CALLIPHORA ERYTHROCEPHALA*)

By P. TATE, *From the Molteno Institute, University of Cambridge*

Recently a sex-linked and sex-limited white-eyed mutant of *Calliphora erythrocephala* has been described (Tate, 1947). While investigating the genetics of this mutation, batches of pupae and mature larvae were kept as reserve material in a refrigerator at a temperature of approximately 4° C. It was found that at this temperature they would continue to develop slowly. The larvae would pupate and the pupae continue to develop so that, if they were removed after varying times in the refrigerator, imagines would emerge more or less quickly according to the time they had been in the cold. The longer they had been in the cold the smaller the percentage of imagines that emerged; but the percentage emergence for batches kept in the cold for 1–2 months was nearly as high as for batches which had been allowed to develop at a temperature of about 21° C. In some cases, when larvae or pupae were put in the cold and allowed to remain there for long periods, a small percentage of imagines actually emerged at the low temperature about 6 months after they had been placed in the refrigerator.

In mutant females which emerged from pupae that had been kept for some time at the low temperature the colour of the eyes at emergence was darker than in females which had emerged from pupae not submitted to a period of cold. In most cases this darkening was one of degree, the colour of the eye of the newly emerged female resembling that of the aged female which had emerged under ordinary conditions of temperature, i.e. an apricot colour. In the females which emerged in the refrigerator, however, the eye colour of the mutant females differed not only in degree but also in quality from the usual colour of the mutant eye. In two batches of imagines which emerged in the cold nearly 6 months after pupation, the eyes of the females were reddish and, when they were teased out in saline and examined microscopically, it was found that definite red pigment granules were present in the secondary pigment cells and probably also in the primary pigment cells; but no brown granules were present. It was not possible to breed from the few red-eyed females which were obtained in this manner. When the darker-eyed females and the males which had emerged after shorter periods at the low temperature were mated, the progeny had eyes of the usual mutant type and the eyes of the newly emerged females were not more yellow than those of females newly emerged from pupae which had not been kept in the refrigerator.

As no red pigment has ever been seen to develop even in very old mutant females at ordinary temperatures, the occurrence of red pigment in females which had emerged in the cold is very interesting, and it is difficult to understand the mechanism of its formation. It indicates that the necessary substrate and enzymes for the formation of pigment must be present in the mutant eye, but that the essential reactions for its formation do not occur. It is possible that the mutant eye is therefore the result of the presence of an 'inhibitor', such as has been postulated for *Drosophila* by Crew & Lamy (1932) and Cochrane (1936–7), which prevents, or interrupts at some point, the normal chains of reactions of pigment

formation. This hypothetical inhibitor may be partially inactivated by the lower temperature so that the formation of pigment can take place to a limited extent. It is curious that it is the red pigment and not the brown that develops under these conditions, as it is the usual sequence that brown or tan pigment precedes red, the two pigments being related to one another as oxidation reduction products (Schultz, 1935; Cochrane, 1936).

In *Drosophila* the eye mutant *blood* (*wbl*) is notable for showing a difference in colour at different temperatures (Ephrussi & Auger, 1938): at 30° C. the eyes are clear yellow but at 19° C. they are red. Ephrussi & Auger (1938) have shown that changing the temperature does not act during larval life, but is most effective during the second quarter of the period of pupal life; and they consider that the temperature does not influence the production of the $v+$ substance, which is a hormone that converts the vermilion pigment (v) to the wild-type pigment, but that the temperature effect is on some other factor, 'the substrate', which is a precursor of the pigment, and which combines with the $v+$ substance.

SUMMARY

1. Pupae and mature larvae of the blow-fly (*Calliphora erythrocephala*) will continue to develop slowly at a temperature of 4° C.

2. White-eyed mutant females which emerge from pupae subjected to the low temperature have, at emergence, eyes of a deeper yellow colour than mutant females newly emerged from pupae which have developed at 21° C.

3. Some flies of the white-eyed mutant strain emerged after 6 months while still in the refrigerator at 4° C. These mutant females had red pigment granules deposited in the secondary pigment cells and probably also in the primary pigment cells. Red pigment granules are never formed in white-eyed mutant females which emerge from pupae kept at 21° C.

REFERENCES

COCHRANE, F. (1936). Observations on eye-colour development in *Drosophila pseudo-obscura*. *J. Genet.* **32**, 183–7.

COCHRANE, F. (1936–7). An histological analysis of the eye-pigment development in *Drosophila pseudo-obscura*. *Proc. Roy. Soc. Edinb.* **57**, 385–99.

CREW, F. A. E. & LAMY, R. (1932). A case of conditioned dominance in *Drosophila obscura*. *J. Genet.* **26**, 351–8.

EPHRUSSI, B. & AUGER, S. (1938). Développement des couleurs des yeux chez la Drosophile: effets de la température sur le mutant *Blood*. *Trav. Sta. Zool. Wimereux*, **13**, 279–86.

SCHULTZ, J. (1935). Aspects of the relation between genes and development in *Drosophila*. *Amer. Nat.* **69**, 30–54.

TATE, P. (1947). A sex-linked and sex-limited white-eyed mutation in the blow-fly (*Calliphora erythrocephala*). *J. Genet.* **48**, 176–91.

STUDIES ON THE SYNAPSIS IN SALIVARY CHROMOSOMES OF HYBRID *CHIRONOMUS* LARVAE

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(With Six Text-figures)

1. Introduction

The fusion of homologous partners in the salivary glands and other large-celled tissues of Diptera has been much discussed in recent times. While bearing some resemblance to the synapsis of homologous chromatids in meiosis, the synaptic phenomenon in salivary chromosomes is complicated by the fact that the fused partners develop during larval life into multivalent structures of enormous size. In normal homozygous larvae the partners retain their intimate association until they undergo histolysis in the pupal stage. In hybrid larvae, owing to structural differences existing between the partners, synapsis may be interrupted in various sections of the chromosomes. However the extent of pairing even in apparently homologous sections is subject to great variation in *Drosophila* as well as in *Chironomus*. Darlington (1937) assumes that this variation may be due to a 'time limit' by which the pairing process is cut short sooner or later in development. It is, however, equally conceivable that the synaptic relationship may not become fixed at any definite period in larval life. The variations in the extent of pairing may reflect changes in the physical constitution of the chromosomes, in the condition of their matrix, or in the state of the nuclear sap. Painter (1941) in his review of structural variations in salivary chromosomes, has stressed our ignorance concerning the changes undergone by these elements in late larval life.

The present investigation was started with the object to determine whether the variations in synaptic affinity of salivary chromosomes in *Chironomus* hybrids bear any relation to the age of the larvae or the temperature at which they were reared. It was hoped that the results might throw some light on the phenomenon of synapsis as well as on the changes undergone by salivary chromosomes during late larval life.

2. Material and methods

The parental strains employed in the crosses both belong to the genus *Chironomus*, group *Thummi*. Strain A was collected in a pool in a Jerusalem garden, while strain B was found in a cistern at Aquabella, 8 km. west of Jerusalem (Judean Hills). At the time of crossing, strain A had been kept in the laboratory for several years, with considerable in-crossing, strain B for over 1 year. Mass-matings had to be employed throughout, since single matings are rarely successful. The parental batches of eggs to be employed in the crosses were reared in large Petri dishes, and a few larvae of each were tested for homozygous salivary chromosomes in order to exclude the possibility of contamination. The flies were collected daily from the dishes and were transferred to cages consisting of a wooden framework (2 m. high, 1 m. long and 0·75 m. deep), covered with cotton mosquito gauze. At the bottom of each cage there was an open Petri dish full of water, so as to enable the

females to deposit the eggs. The batches of hybrid eggs* were transferred to Petri dishes 15 cm. wide and 4 cm. high and provided with powdered mud for tube-building and with filtered water from a balanced pond. The dishes were kept in constant temperature chambers of 13, 20, 25 and 30° C. respectively. The fluctuations of the temperature within these chambers were on the whole within the limits of ±1·5° C. The larvae were fed on a mixture of powdered Quaker Oats and dried egg. Under these culture conditions larvae are not more crowded than they may be found on the bottom of any pond in the open. However, competition seems to be severe, since the prepupal stage is not reached by all larvae at the same time, and since the animals persist in this stage for extended periods. Beginning with the stage marked by the appearance of several prepupae, prepupae were removed at intervals from the dishes, and smears of the salivary glands were made at room temperature. The prepupae showed great variation in length, but measurements of the head capsules indicated that all belonged to the same instar. The glands were dissected out in a drop of Ringer's solution and were left for 10 min. in 45% acetic acid and stained for 10 min. in acetocarmine before crushing. The preparations were made permanent by transferring through 95% alcohol into Venetian turpentine.

The peculiarities of synapsis in this hybrid are described in a previous paper (Goldschmidt, 1942*a*). The three long chromosome pairs are invariably non-synapsed in the central region. This is characterized by a sub-median 'puff' in strain B which is absent in strain A, besides other structural differences. The ends exhibit synapsis to a varying extent. The small satellite chromosomes are usually synapsed at one end and unpaired at the other (Fig. 5).

Since it is impossible to record the synaptic condition of every single band in large numbers of nuclei, the following scheme of counting was adopted: The chromosomes were considered as made up of two arms,† connected by the central region, which, in the long chromosomes of strain B, bears the 'puff'. In the small satellite chromosome the central region is marked by fluffy heterochromatic bands. For each chromosome in each nucleus the synaptic condition of the homologues in both arms was recorded. Synapsed, half-synapsed and non-synapsed arms were distinguished. An arm was considered as synapsed when showing fusion of the homologues in a greater or smaller number of their bands (cf. Fig. 5, R I, L II, R II, L III, L IV). When the homologues were in loose contact, only connected by a narrow bridge of chromatic material, the arm was considered as half-synapsed (cf. Fig. 5, L I). When the homologues were perfectly free from each other the arm was considered as non-synapsed (cf. Fig. 5, R III, R IV). The number of non-synapsed arms in each nucleus was counted, a non-synapsed arm being scored as 1, a half-synapsed arm as ½. In order to avoid unconscious selection, only well-preserved nuclei in which the characteristic bands (land-marks) of each chromosome could be identified, were used. No more than eight nuclei were used in any preparation.

3. THE EFFECT OF AGE

Throughout the cultures examined, synapsis was found to diminish with rising age of the larvae.

Tables 1 and 1A show the results obtained from six samples taken at different times

* Each egg mass being enclosed in its own sausage-shaped jelly there is no difficulty in keeping the offspring of each mother separate.

† In mitoses, all four chromosomes are V-shaped (Goldschmidt, 1942*b*).

from a culture kept at 20° C. Table 1 shows, for each sample, the numbers of nuclei containing various numbers of non-synapsed arms. The means and their standard errors were obtained from these figures in the usual manner. Table 1A summarizes the results of Table I and includes, in addition, the percentages of non-synapsed arms in the right arms

Table 1. *Culture* A 36, 9 *November* 1943, *kept at* 20° C.

Age in days	Number of nuclei with 1, 1·5, 2, ..., unpaired arms													
	1	1·5	2	2·5	3	3·5	4	4·5	5	5·5	6	6·5	7	7·5
34	7	—	13	1	2	—	—	—	—	—	—	—	—	—
52	8	2	13	4	7	—	1	—	—	—	—	—	—	—
63	3	1	4	1	7	—	1	1	—	2	1	—	1	—
77	1	—	4	1	4	3	6	3	1	2	2	—	—	—
90	—	—	—	1	2	4	4	3	2	2	2	2	1	—
98	—	—	2	1	1	2	3	2	1	2	3	2	—	1

Table 1A. *Culture* A 36, 9 *November* 1943, *kept at* 20° C.

Age in days	No. of larvae	No. of nuclei	% non-synapsis in III R	% non-synapsis in chromosomes I and II	Total % of non-synapsis in all chromosomes	Mean no. of non-synapsed arms per nucleus	Standard error	X	$P<$
34	5	23	63	5	23	1·804	±0·126		
								1·40	0·17
52	11	35	69	8	26	2·057	±0·129		
								2·84	0·01
63	9	22	77	28	39	3·114	±0·349		
								1·34	0·19
77	11	27	94	33	46	3·685	±0·245		
								2·48	0·02
90	10	23	96	44	57	4·565	±0·256		
								0·0815	0·94
98	9	20	100	48	58	4·600	±0·345		

of chromosome III and the percentages of non-synapsed arms in chromosomes I (L. and R.) and II (L. and R.).

Fig. 1 shows graphically the percentages of non-synapsis in R III, the percentages of

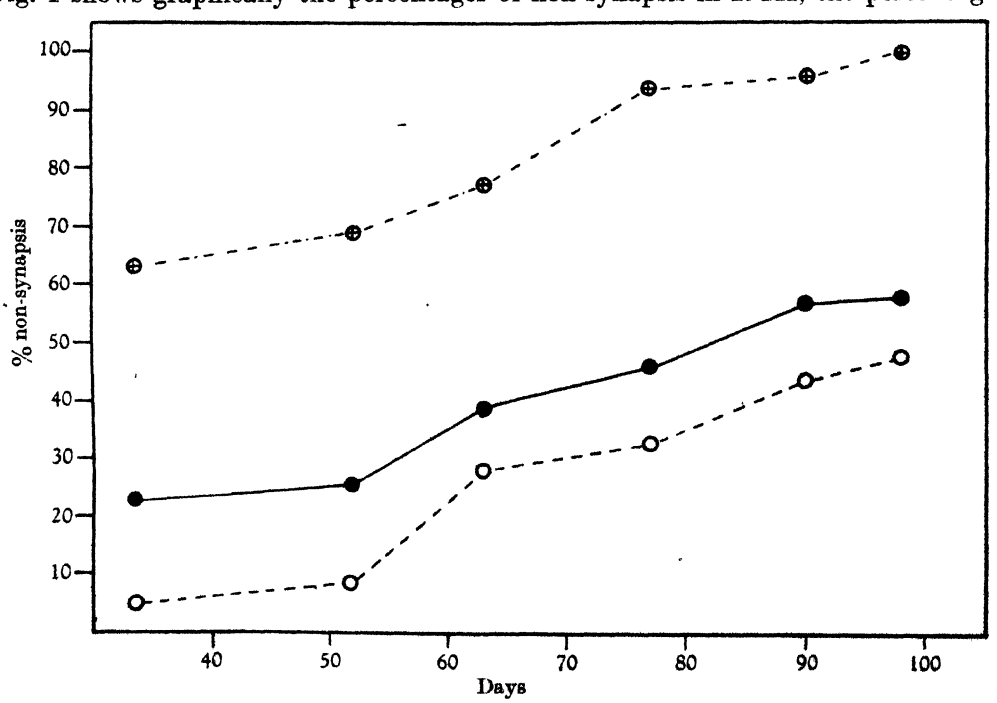


Fig. 1. Diagram showing the relation between age and non-synapsis in culture A 36 kept at 20° C. ⊕, right arm of chromosome III; ○, arms of chromosomes I and II; ●, arms of all four chromosomes.

non-synapsis in chromosomes I and II, as well as the total percentage of non-synapsis, for each sample.

It is seen that the right arm of the third chromosome is often unsynapsed even in young larvae and reaches 100 % non-synapsis in old ones. The arms of chromosomes I and II exhibit great synaptic affinity at early stages (5 % loose arms), while in old larvae they show 48 % of non-synapsis. The total percentage of loose arms rises from 23 % at 34 days of age to 58 % at 98 days. As seen from Table 1A, two of the differences between consecutive stages are significant, corresponding to values of P below the 0·05 level. Since all changes are in the same direction there can be no doubt as to the overwhelming significance of the effect as a whole.

Table 2. *Culture* A 30, 18 *October* 1943, *kept at* 13° C.

Age in days	Number of nuclei with 1, 1·5, 2, 2·5, ..., unpaired arms														
	1	1·5	2	2·5	3	3·5	4	4·5	5	5·5	6	6·5	7	7·5	8
49	—	—	10	7	4	6	4	2	—	—	—	—	—	—	—
56	1	1	11	8	13	6	9	4	2	2	2	3	—	—	—
65	—	—	13	3	8	2	4	2	6	3	3	2	4	1	—
84	1	—	1	2	1	4	6	4	10	11	15	8	5	5	—
94	—	—	1	1	2	2	4	—	9	2	3	6	10	6	2
112	—	—	—	—	—	—	1	—	3	1	1	4	6	5	1

Table 2A. *Culture* A 30, 18 *October* 1943, *kept at* 13° C.

Age in days	No. of larvae	No. of nuclei	% non-synapsis in III R	% non-synapsis in chromosomes I and II	Total % of non-synapsis in all chromosomes	Mean no. of non-synapsed arms per nucleus	Standard error	X	$P<$
49	7	33	85	19	36	2·894	±0·140	2·35	0·02
56	11	62	96	31	43	3·403	±0·165	1·94	0·06
65	11	51	98	42	50	3·971	±0·243	4·83	0·000,01
84	14	73	97	68	67	5·370	±0·159	1·51	0·14
94	10	48	94	73	72	5·783	±0·222	2·47	0·02
112	4	22	98	84	82	6·546	±0·215		

Tables 2 and 2A and Fig. 2 were obtained from a culture kept at 13° C. The three curves in Fig. 2 start at higher levels than the corresponding curves in Fig. 1, but are similar in trend. However, the right arm of chromosome III shows 85 % of non-synapsis in the beginning and subsequently fluctuates between 94 and 98 % without reaching the 100 % level. Some of the differences between the means of consecutive samples are highly significant (cf. Table 2A).

There is thus an obvious effect of age on the amount of synapsis in the hybrid, the number of synapsed arms (and most probably the total number of synapsed bands) decreasing with rising age of the larvae.

4. THE EFFECT OF TEMPERATURE

The marked age effect renders the estimation of a possible temperature effect a most difficult matter. For while all larvae employed were in the prepupal stage, this instar is of very different duration at various temperatures and thus the 'absolute age' of the larvae cannot be determined exactly. The time during which prepupae can be obtained from moderately crowded cultures varies from 18–20 days at 25° and 30° to 60–70 days at 13° C. Culture conditions for aquatic larvae feeding on a variety of micro-organisms which in their turn depend upon the food substances added to the water are obviously

far from standardized, especially when the differential effect of temperature on the various food organisms comes into play. Bearing in mind these reservations, it is nevertheless of some interest to compare the curves obtained from cultures at various temperatures.

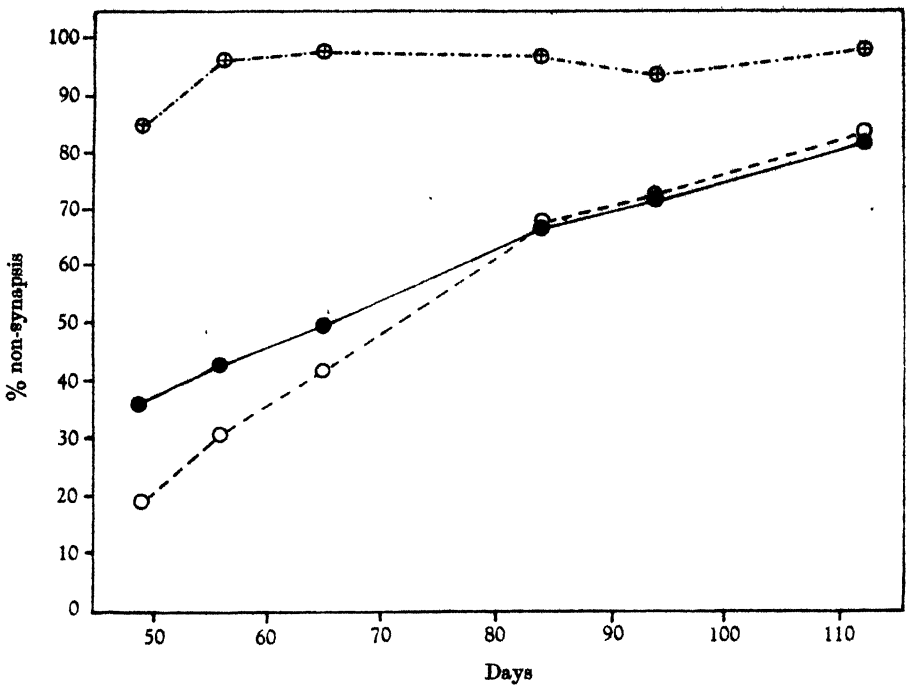


Fig. 2. Diagram showing the relation between age and non-synapsis in culture A 30, kept at 13° C. ⊕, right arm of chromosome III; ○, arms of chromosomes I and II; ●, arms of all four chromosomes.

Tables 3 and 3A were obtained from a culture kept at 25°, Tables 4 and 4A from a culture kept at 30° C.

Table 3. *Culture* A 25, 13 *August* 1943, *kept at* 25° C.

Age in days	Number of nuclei with 1, 1·5, 2, ..., unpaired arms													
	1	1·5	2	2·5	3	3·5	4	4·5	5	5·5	6	6·5	7	7·5
35	11	10	23	11	10	3	4	2	1	—	—	—	—	—
52	1	2	7	5	6	6	7	5	4	2	3	2	4	3

Table 3A. *Culture* A 25, 13 *August* 1943, *kept at* 25° C.

Age in days	No. of larvae	No. of nuclei	% of non-synapsis in all chromosomes	Mean no. of non-synapsed arms per nucleus	Standard error	X	$P<$
35	16	75	28	2·266	±0·108	7·08	10^{-9}
52	19	57	51	4·078	±0·232		

Table 4. *Culture* A 34, 2 *November* 1943, *kept at* 30° C.

Age in days	Number of nuclei with 2, 2·5, 3, ..., unpaired arms												
	2	2·5	3	3·5	4	4·5	5	5·5	6	6·5	7	7·5	8
30	4	2	5	1	1	1	2	1	—	1	—	—	—
35	5	—	1	3	2	1	3	—	1	1	—	—	—
40	1	—	3	—	3	1	—	2	1	—	—	—	—
48	1	—	2	—	1	2	3	2	2	—	4	1	1

Table 4A. *Culture* A 34, 2 *November* 1943, *kept at* 30° C.

Age in days	No. of larvae	No. of nuclei	% of non-synapsis in all chromosomes	Mean no. of non-synapsed arms per nucleus	Standard error	t*	$P<$†
30	7	18	43	3·444	±0·308	—	—
35	9	17	47	3·736	±0·343	—	—
40	8	11	51	4·046	±0·361	2·35	0·05
48	5	19	67	5·394	±0·370		

* t was obtained by Fisher's formula (1944, p. 122).
† For the difference between the uppermost and lowermost values of the series $t=3·92$ corresponding to a value of P smaller than 0·001.

Fig. 3 is based on the results of Tables 1–4 and represents the changes in the total percentage of non-synapsis in the four cultures kept at 20, 13, 25 and 30° C. respectively. For the cultures kept at 13, 20 and 30° C., the regression lines were calculated from the

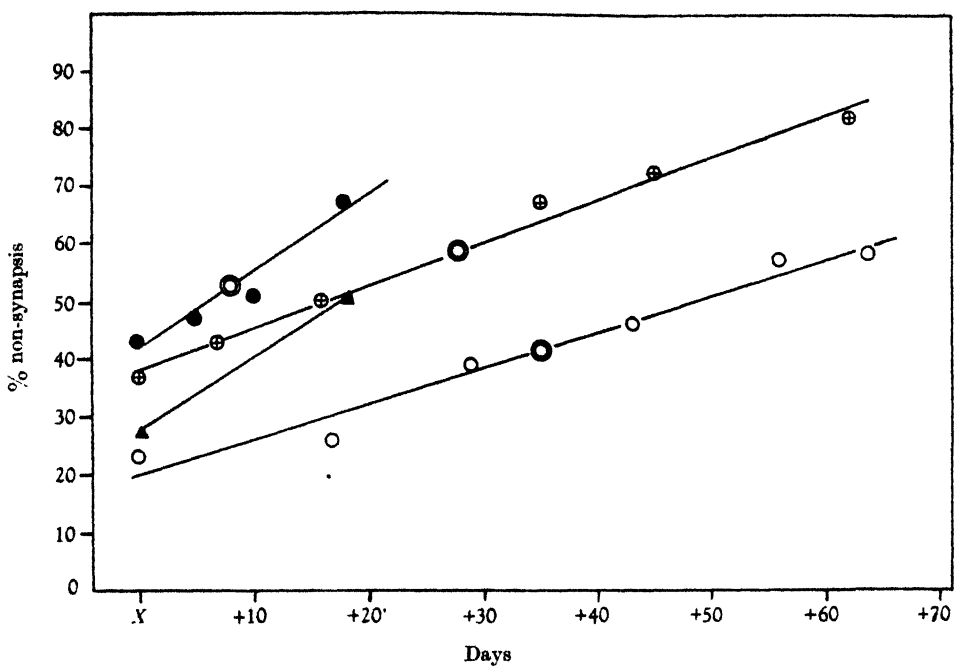


Fig. 3. Diagram showing the regression of non-synapsis on age in four cultures kept at different temperatures. ⊕, at 13° C.; ○, at 20° C.; ▲, at 25° C.; ●, at 30° C.; ◎, mean values; X marks the time at which prepupae first appeared in each culture.

points available. From the culture kept at 25° C. only two samples were investigated, and the line was obtained by connecting the two resulting points. In order to allow of a survey of the course of events in the four cultures, the initial points of all four lines are arranged in one vertical line. Since these points mark the time at which prepupae first appeared in the cultures, the abscissa represents the number of days which elapsed in each culture after this initial date. The slopes of the heat culture (30° C.) and of the cold culture (13° C.) are seen to be steeper than that of the culture kept at 20° C. For the heat culture this difference in slope cannot be properly assessed without an exact knowledge of the temperature coefficient of growth. In the case of the cold culture the temperature effect on growth should tend to lessen the slope. Hence, the steeper slope of the 13° C.

line as compared with the 20° C. line at once suggests a specific effect on synapsis of the cold treatment. If the rate of non-synapsis at the initial points of each line is plotted against temperature, the 'curve' obtained has a minimum at a moderate temperature (20° C.), while rising at either end (Fig. 4). The significance of the differences between the initial points of the cultures may be gauged from Table 5.

Table 5. *Initial points of cultures kept at different temperatures*

Temperature ° C.	Culture	Mean no. of non-synapsed arms per nucleus	Standard error	X	P
13	A 30 18 October 1943	2·894	±0·140	5·78	10^{-8}
20	A 36 9 November 1943	1·804	±0·126	2·79	0·01
25	A 25 13 August 1943	2·266	±0·108	3·61	0·001
30	A 34 2 November 1943	3·444	±0·308		

When plotting the mean non-synapsis values (the mean of the percentages of all samples of each culture) against temperature a similar U-shaped curve is obtained (Fig. 4), though with a minimum at 25° C.

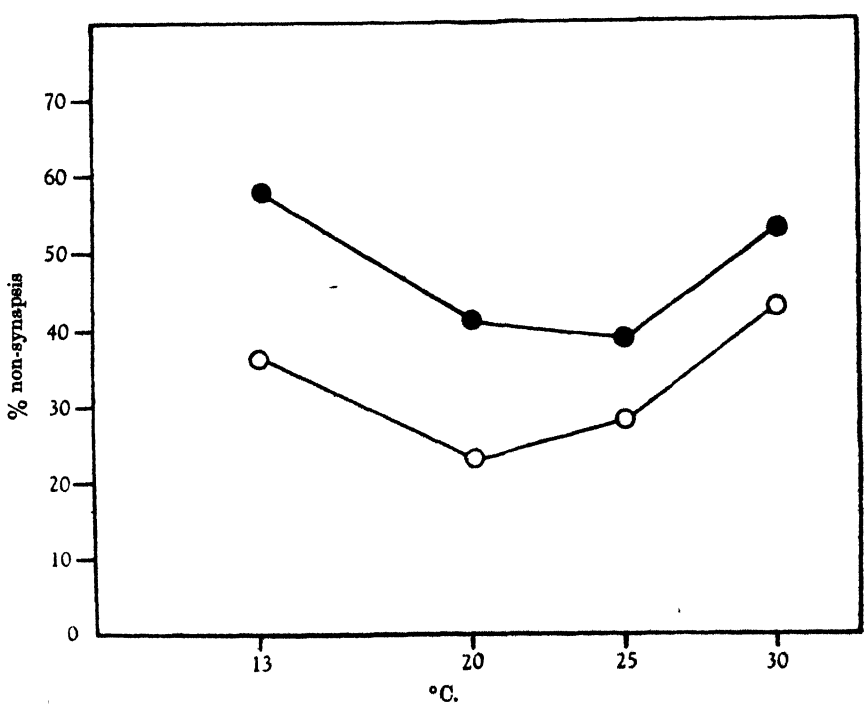


Fig. 4. Diagram showing the relation of non-synapsis to temperature (cf. Fig. 3). ○, initial values; ●, mean values of four cultures kept at different temperatures.

5. Data from additional cultures

The data available are not sufficient to allow of a full estimate of the variation exhibited by different cultures kept at the same temperature. However, the agreement of the

regression line calculated for the heat culture A 34 with data from other heat cultures was tested, utilizing five counts obtained from three different cultures kept at 30° C. (cf. Table 6).

Table 6. *Control cultures kept at* 30° C.

Culture	Age in days	No. of larvae	No. of nuclei	Total % of non-synapsis in all nuclei
A 15, 28 July 1943	40	8	27	68
A 18, 4 August 1943	37	8	31	49
	48	8	28	72
A 37, 10 November 1943	29	9	23	40
	42	11	32	65

The coefficient of regression of culture A 34 is 1·33 with a standard error of ±0·236 (calculated by Mather's formula, p. 115). The three cultures A 15, A 18 and A 37 combined gave a coefficient of regression = 1·82 with a standard error of ±0·420. On estimating the difference between the two coefficients a value of $t = 1·01$ is obtained, corresponding to a probability of between 0·5 and 0·3 for 5 degrees of freedom (cf. Mather, p. 119). This indicates a good agreement between the two sets of data. The coefficient of regression obtained from all four cultures in common is 1·62 ± 0·280, corresponding to a yet more rapid increase in non-synapsis than that observed in culture A 34.

Table 7. *Culture* A 35, 9 *November* 1943, *kept at* 13° C.

Age in days	Number of nuclei with 1, 1·5, 2, ..., unpaired arms														
	1	1·5	2	2·5	3	3·5	4	4·5	5	5·5	6	6·5	7	7·5	8
75	1	2	12	6	3	5	2	1	2	—	—	—	—	—	—
90	—	1	6	5	14	1	4	1	—	—	—	—	—	—	—
112	—	1	6	1	6	8	8	10	8	4	2	1	—	—	—
131	—	—	3	3	5	8	10	10	6	1	3	—	1	—	—
152	—	—	—	—	5	5	7	12	4	7	13	9	6	4	1
181	—	—	—	—	1	2	1	2	3	1	3	5	3	2	2
188	—	—	—	—	—	—	1	5	3	2	4	7	15	8	6
195	—	—	—	—	—	—	—	—	1	3	5	5	9	1	1

Table 7A. *Culture* A 35, 9 *November* 1943, *kept at* 13° C.

Age in days	No. of larvae	No. of nuclei	Total % of non-synapsis in all chromosomes	Mean no. of non-synapsed arms per nucleus	Standard error	X	P
75	8	34	34	2·706	±0·168	0·82	0·42
90	9	32	36	2·875	±0·123	5·58	0·000,0001
112	12	55	50	3·990	±0·157	0·23	0·82
131	14	50	51	4·040	±0·152	6·06	10^{-6}
152	17	73	67	5·336	±0·154	1·58	0·12
181	10	25	73	5·841	±0·280	2·27	0·03
188	16	51	82	6·568	±0·154	0·33	0·75
195	7	25	81	6·500	±0·140		

Tables 7 and 7A contain the results of counts made in a culture (A 35, kept at 13° C.), which showed a much retarded growth as compared with culture A 30 (kept at 13° C., cf. Tables 2 and 2A). The first prepupae appeared as late as 75 days after hatching. At this stage non-synapsis amounted to 34 % as compared with 36 % in the first sample of A 30 (49 days old), while a final value of 82–81 % is reached corresponding to 82 % in the last sample of A 30. There is thus a striking coincidence between the values of non-synapsis in the two cultures in spite of the discrepancy in developmental rates.

6. Short temperature treatment

Larvae of a culture kept at 25° C. were transferred to temperatures of 30 and 8° C. respectively and smears were made after 48 hr. A preliminary examination of these smears did not reveal any marked difference in the synaptic conditions of either section from that of the controls, left at 25° C.

7. Discussion

While in homozygous *Chironomus* larvae synapsis of the giant chromosomes is complete from the beginning of the third instar, the results presented indicate clearly that the extent of synapsis in hybrid larvae is inversely proportional to age. It may be concluded that with rising age conditions in the cells of the gland change in a manner which causes the homologous partners to dissolve their union progressively. There is thus no time limit of pairing, the relationship of the partners being subject to change throughout larval life.*

In taxonomic cytology the extent of synapsis is often regarded as an indicator of the relationship of the parental races. It should be realized that larvae of different ages may yield entirely different salivary pictures (cf. Figs. 5, 6).

While the age-effect on synapsis appears to be well established by the present findings, the effect of temperature needs further confirmation. A *Drosophila* hybrid (*D. simulans* × *melanogaster*, or *D. virilis* × *americana*, cf. Muller, 1940) which can be kept under standard food conditions, might constitute a more favourable material than the present hybrid. The preliminary curve obtained from the initial points and from the mean values of the cultures kept at different temperatures shows a minimum at moderate temperatures (20–25° C.) and rises on both sides. This result is in good agreement with the general course of the temperature curves of crossing-over and of chiasma frequency.† (Cf. Plough, 1917, 1921; White, 1934; Smith, 1936.)

It is of some interest to note that temperature extremes produce a rise in crossing-over (i.e. chromosome breakage) but a decrease in the synapsis of giant chromosomes. Since plasma viscosity in some types of cells may also exhibit a minimum at moderate temperatures (Heilbrunn, 1928; Belehradek, 1935), it appears possible that viscosity changes (in the chromosomes themselves or in the surrounding medium) may be responsible for the temperature effects on both chiasma frequency and synaptic behaviour.

The physico-chemical causes underlying synapsis in both meiotic and salivary chromosomes are still quite undefined, and the theories that have been advanced in recent times in connexion with this phenomenon are all highly speculative:

(1) Delbrueck (1941) proposes that the attraction may be an electrochemical effect due to the formation in each partner of negatively charged semiquinone radicals at intervals. One-electron resonance bonds would then be formed between charged and uncharged

* The possibility of an alternative interpretation should not be overlooked. It might be supposed that different larvae are characterized by different synaptic tendencies from the outset and that the synaptic conditions become 'fixed' at a certain stage of development. Larvae with more intimate pairing might mature earlier than larvae with a relative lack of synapsis, thus producing the observed effect. However, since all larvae are derived from the same two inbred parental races it is most unlikely that there should exist among them vast differences in genic make-up, resulting in the widely divergent synaptic states, which were observed. It is by far more plausible that the partners in all larvae change their relationship progressively.

† The effect of age on chiasmata and crossing-over appears to differ in different organisms. In mice and rats chiasmata are reported to decrease with age (Bryden, 1933), whereas in *Drosophila* the number of cross-over offspring, plotted against the age of the mother, yields a curve with several maxima and minima (Plough, 1917).

radicals of both partners. The sterical arrangement pattern of the charged radicals would ensure the exact 'fit' of homologous sections. According to this theory a change in the redox situation of the nucleus is required to transform attraction into repulsion by an oxidation of the charged radicals.

(2) Bernal (1940) points out that specific attraction might be exercised by nucleoprotein units of specific lengths. Homologous units might possess electrostatic charges of equal sign and still attract one another by Langmuir-Levene forces exerted by each unit

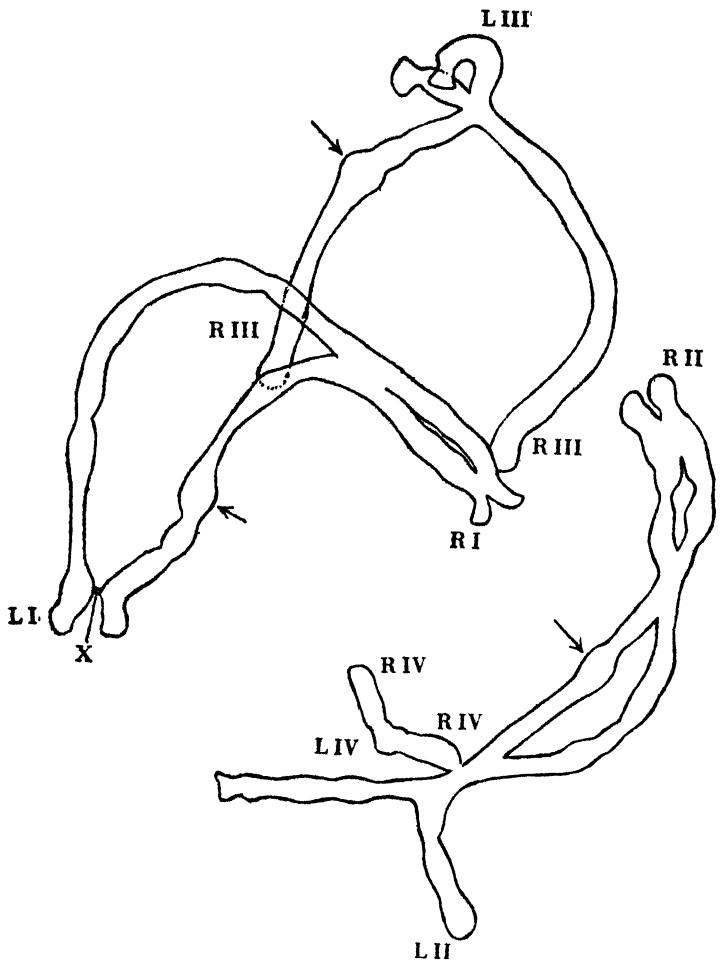


Fig. 5. Camera-lucida diagram of salivary chromosome smear showing synapsis. (A 35, 75 days old, cf. Table 7). Arrows indicate the 'puffs' characterizing the central regions in chromosomes of strain B. X marks a region of half-synapsis (scored as ½). ×930.

on the ionic sphere of its partner. According to this theory a slight change in the *p*H or the concentration of the medium would be sufficient to convert attraction into repulsion.

(3) Fabergé (1942) expounds the theory of chromosome attraction by the Guyot-Bjerknes effect. This is a hydrodynamic phenomenon resulting in the attraction (or repulsion) of pulsating (or oscillating) spheres. Fabergé shows that a rise in the viscosity of the nuclear fluid may restrict the range of vibration frequencies capable of producing the effect under cell conditions.

While the present results give no information as to the changes in *p*H or redox potential of the salivary gland nuclei, they would seem to indicate that viscosity changes, at any rate, are involved. It could be noticed that on the whole chromosomes with synapsed ends tend to stretch more on smearing than do unsynapsed chromosomes. The former tend to appear long and thin, the latter are usually extremely thick and short (cf. Figs. 5, 6).

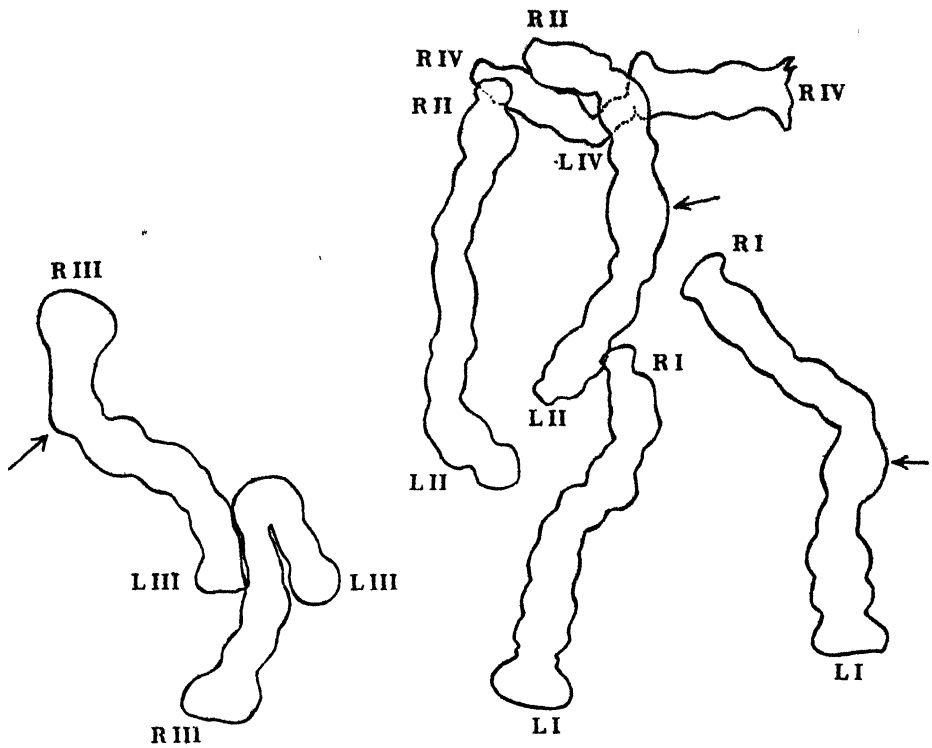


Fig. 6. Camera-lucida diagram of salivary chromosome smear showing complete lack of synapsis except for the left end of chromosome IV (A 35, 188 days old, cf. Table 7). Arrows indicate the 'puffs' of strain B. ×930.

This difference may be related to changes in the viscosity of the nuclear sap or the chromosome matrix or both and may thus be correlated with the differences in synaptic behaviour.* On the basis of Fabergé's (and possibly also of Bernal's) theory, viscosity changes in the salivary gland nucleus might account for the observed changes in synapsis.

While all counts were made on smears, examination of sectioned material revealed that non-synapsed chromosome portions do not lie side by side, but are usually widely separated. It may be concluded that non-synapsed portions of chromosomes actually repel one another.

Summary

1. The extent of synapsis in hybrid *Chironomus* larvae decreases with rising age.

2. There is less synapsis in hybrid larvae grown at high (30° C.) and at low (13° C.) temperatures than in larvae kept at moderate temperatures (20–25° C.).

* It must be remembered, however, that this effect was noticed after fixation with acetocarmine and that the relations between viscosity and elasticity are complicated (cf. Heilbrunn, 1928).

3. In sectioned material of hybrid glands non-synapsed portions tend to be widely separated as though repelling one another.

4. The results are discussed in the light of some recent theories on the nature of synaptic attraction.

I wish to thank Dr A. Bak, Prof. F. S. Bodenheimer and Dr K. Reich for kindly putting their constant-temperature chambers at my disposal; Dr G. Haas and Dr K. Reich for their kind interest in the work, and Dr W. Koch and Dr M. Schiffer for their friendly advice in matters of statistics. I am also greatly obliged to Prof. J. B. S. Haldane for reading and criticizing the manuscript.

REFERENCES

BELEHRADEK, J. (1935). Temperature and living matter. Gebr. Borntraeger. Berlin.

BERNAL, J. D. (1940). Structural units in cellular physiology. *Cell and Protoplasm.* The Science Press.

BRYDEN, W. (1933). The relation of age to frequency of chiasma formation in the Wistar rat. *J. Genet.* **27**.

DARLINGTON, C. D. (1937). Rec. Adv. Cytology, London.

DELBRUECK, M. (1941). A theory of autocatalytic synthesis of polypeptides and its application to the problem of chromosome reproduction. *Symp. Quant. Biol.* **9**.

FABERGÉ, A. C. (1942). Homologous chromosome pairing. The physical problem. *J. Genet.* **43**.

FISHER, R. A. (1944). *Statistical methods for research workers.* London.

GOLDSCHMIDT, E. (1942*a*). The pattern of salivary gland chromosomes. *J. Hered.* **33**.

GOLDSCHMIDT, E. (1942*b*). Cytological studies in *Chironomidae*. Summary of Thesis for the degree of Ph.D., The Hebrew University, Jerusalem.

HEILBRUNN, L. V. (1928). The colloid chemistry of protoplasm. Gebr. Borntraeger. Berlin.

MATHER, K. (1943). *Statistical Analysis in Biology.* London: Methuen.

MULLER, H. J. (1940). Bearing of the *Drosophila* work on systematics. *The New Systematics.* Oxford.

PAINTER, T. S. (1941). An experimental study of salivary chromosomes. *Symp. Quant. Biol.* **9**.

PLOUGH, H. H. (1917). The effect of temperature on crossing over in *Drosophila*. *J. Exp. Zool.* **24**.

PLOUGH, H. H. (1921). Further studies on the effect of temperature on crossing-over. *J. Exp. Biol.* **32**.

SCHRADER, F. (1944). *Mitosis.* Columbia University Press.

SMITH, H. F. (1936). Influence of temperature on crossing-over in *Drosophila*. *Nat.* **138**.

STERN, C. AND RENTSCHLER, V. (1936). The effect of temperature on the frequency of somatic crossing-over in *Drosophila*. *Proc. Nat. Acad. Sci.* **22**.

WHITE, M. J. D. (1934). The influence of temperature on chiasma frequency. *J. Gen.* **29**.

A QUANTITATIVE STUDY OF PETAL SIZE AND SHAPE IN *SAXIFRAGA GRANULATA* L.

By E. M. MARSDEN-JONES and W. B. TURRILL

(With Plates 8–10)

Saxifraga granulata L. (*Sp. Pl.* 403, 1753) is a variable species with a wide distribution through Scandinavia, Central Europe, the Iberian Peninsula, Italy, Sicily, Morocco, and (as the subsp. *graeca*) the Balkan Peninsula. It is rather common locally and generally distributed in England, but, becoming scarce north of the Forth and Clyde, it does not extend in Scotland northwards beyond Aberdeen and the Moray Firth. Numerous paramorphs (intraspecific groupings) are described by Engler & Irmscher (*Das Pflanzenreich*, **4**, 117, 244–56, 1919).

Sixteen years ago a large population of *S. granulata* was found near the Hog's Back, Guildford district, and was investigated periodically for a number of years. The Hog's Back itself is an outcrop of chalk extending approximately east and west to the west of Guildford. Parallel with this ridge and to the south of it there outcrop a series of beds of Cretaceous age, including Upper Greensand and Gault and divisions of the Lower Greensand. These rocks are lithologically very different one from another and give rise to soils which have been, at least in part, formed *in situ* and are largely immature. In their chemical and physical features, especially in their permeability, they are very distinct and result in the occurrence of different floras and plant communities which, following the rock outcrops, run approximately east and west in relatively narrow strips. The population of *S. granulata* was on the Folkestone Beds, which are of sandy nature and the highest beds in this area of the Lower Greensand. It was studied for a distance of nearly two miles along a strip less than a quarter of a mile wide.

The autecology of the species is not dealt with in this paper which is concerned only with certain studies made on the flowers. During a visit to the above defined area on 28 May 1930, when the species was at full anthesis, we were much struck by the variety in the flowers, particularly in the size and shape of the petals. A large number of plants was examined and a series of twenty were selected as showing the range of variations in petal characters, dug up, and transplanted to the experimental ground at Potterne, Wilts. (on Upper Greensand soil). These stock plants were a selected and not a random sample. They remained constant for five years for petal shape and, with the F_1 and F_2 families raised from them, were collected for scoring in May 1935. Independently of these stock plants, a random sample of fifty plants was analysed for petal characters and the results of this analysis are given separately below. We know that *S. granulata* can be crossed with other species of the genus with sometimes unexpected results, but it is important to note that in the Hog's Back population there is no indication of any hybridization with another species. All the samples and families considered in this paper are definitely determined as belonging to the taxonomic species *S. granulata*. The possibility of *S. granulata* itself being a polyploid is referred to below.

The purpose of this paper is to indicate, as far as possible, the factors involved in petal size and shape. Some preliminary considerations are necessary in order to understand the

complexity of the problem and the limitations of the methods employed. The descriptive statements given here refer entirely to the Hog's Back population and to plants derived from this.

S. granulata is a perennial with globular scaly bulbs crowded at or slightly below ground-level. The flowering stems are erect and vary from one to half a dozen or more according to the luxuriance and age of the plant growing *in situ* without disturbance. The degree of branching of the flowering stems, and consequently the number of flowering stems produced, also varies within wide limits. Small plants may develop only one unbranched flowering stem producing only two flowers. At the other extreme, wild plants with seven and cultivated plants with eight lateral branches to one flowering stem have been observed. The maximum number of flowers counted on one flowering stem and its branches is twenty-six in wild and thirty-six in cultivated material. The structure of the flowering stem is somewhat complicated. The main axis ends in a flower which is the oldest and often has been pollinated and has shed its petals before the flowers on the lateral branches open. The branches arising laterally on this main axis of definite growth average three to four and arise in racemose or apparently racemose sequence from the axils of bracts (the lowest more or less leaf-like) with sometimes 1/3, sometimes 2/5 phyllotaxis. Every branch ends in a flower and has a sterile α bracteole and a fertile β bracteole; in the axil of the latter a flower usually comes to maturity. In the more luxuriant plants every branch or some of the branches may repeat more or less the structure of the main axis. There is no doubt as to the basic cymose (definite) structure of the flowering stem as a whole or of the cymose nature of the final branch endings. The acropetal sequence of the branches apparently lateral to the main axis is less easy to determine for the following reasons. There is a marked tendency for all first flowers of all lateral branches to be at the same stage of anthesis irrespective of the sequence of the branches; an increased number of flowers (beyond two) per branch of second order may slightly retard the anthesis of the first flower of the branch (partial inflorescence); the lowest branches, especially if the subtending bract be more of foliage leaf form than the bracts subtending upper branches, are occasionally retarded in growth, as if they had arisen from buds which would have remained dormant but for some, perhaps nutritional, stimulus. The conclusion is reached that the flowering stem as a whole is compound and shows a sequence of cymose → racemose → cymose structure. Measurements made of flowers on a large number of plants showed there were no regular and significant differences in length/breadth ratios or shape between the petals of flowers arising at different positions in the whole inflorescence. In particular, the first (i.e. terminal) flowers of the lateral (second order) branches were very uniform on the same plant and, as far as possible, these were the ones used for measurement.

The flower in *S. granulata* is approximately actinomorphic. The calyx, corolla, and androecium are epigynous. The five petals have a 2/5 aestivation with the overlapping sepal, no. 2 in the whorl, adaxial, but show very little overlap even in young flower buds. The five petals, which are easily and clearly detachable, have a 3/5 aestivation (at least most often) with the overlapped petal (no. 4 in the whorl) abaxial. At full anthesis the petals may or may not overlap, according to their shape and size. The ten stamens are in two whorls and obdiplostemonous, a condition best determined in the bud. The gynoecium, with normally two stigmata (three have been several times observed), is, in lateral flowers, usually more or less oblique to the bract axis plane. The flowers are markedly

protandrous. There is sometimes a slight difference in size and shape between the petals of a single normal flower. One typical example indicates the range in mm.:

		Petals				
127/3:	Length	11·5	12·0	12·0	12·5	11·5
	Breadth	6·5	6·5	6·0	7·0	6·5
	Height above base of widest part	8·5	8·5	8·0	8·5	8·0

The size, and to some extent the shape, of the petals varies with the age of the flower. There is usually an increase in the ratio of length to breadth from young buds to just before anthesis when there is a fall in the ratio value to an intermediate figure. As an example, measurements are here given for twelve flowers from one plant (127/14), arranged in order of increasing age of the flowers, to the nearest 0·25 mm.

Flowers (youngest first)	Length (L)	Breadth (B)	Ratio (L/B)
1	2·50	1·50	1·67
2	3·50	2·00	1·75
3	4·00	2·00	2·00
4	5·50	2·75	2·00
5	6·50	3·25	2·00
6	7·00	3·25	2·16
7	8·00	3·50	2·29
8	9·00	4·00	2·25
9	10·00	4·25	2·35
10	11·00	5·00	2·20
11	11·50	5·50	2·09
12	12·00	6·00	2·00

Bearing in mind the above behaviour and individual (ontogenetic or positional) variations the following rules have been observed in selecting petals for measurements in the samples and families analysed in this paper. Chosen were:

(1) well-formed flowers (exceptions in 'abnormals' are noted below);

(2) flowers from branches of second order (when present and in satisfactory condition);

(3) flowers at full anthesis, preferably at the stage after dehiscence of the anthers but before full maturation of the stigmata;

(4) best developed petal in the flower.

Measurements were made under a dissecting microscope from flattened petals to 0·5 mm. Those for length and breadth were easily made, but the position of the widest part above the base was sometimes less satisfactorily determined. The petals are always more or less gradually narrowed in the lower half though not into a distinct claw. They also narrow towards the apex, but more abruptly and usually in the uppermost sixth or third of their length. The widest part is sometimes clearly discernible, but a slight asymmetry occasionally makes it difficult to determine within 1–2 mm. Further, when the petals are long and narrow their sides become parallel or approximately parallel a few mm. above the base to a little below the apex. This parallel-sided portion is the widest part of the petal and measurement above the base has been taken at the centre of this widest portion. With these difficulties in mind allowance must be made for a greater range of 'error' in the measurements for widest part than in those for length and breadth.

The above remarks apply *in toto* to 'normal' plants and 'normal' flowers, that is to plants whose flowers have the formula K5 C5 A5+5G ($\bar{2}$), on the commonly accepted interpretation of the gynoecium. There are, however, in the Hog's Back population and in families bred from plants of this population, two abnormalities which are frequently associated with modifications of the petals and in certain of the families analysed below

have to be taken into account in considering petal size and shape. These are, for convenience, referred to as 'females' and 'abnormals' respectively.

Females. The term 'female' is here used to indicate flowers (and by extension plants) producing no viable pollen but with the gynoecium normal. These functional female flowers can equally correctly be described as male sterile, since though stamens are produced with obvious filaments and anthers, they are smaller than usual, and the anthers are empty of pollen grains and their loculi have often collapsed. There are grades of 'femaleness' or, in other words, intergrades between the hermaphrodite and female condition, though in the Hog's Back population of *S. granulata* and families raised from this the plants mostly have either only fully hermaphrodite or functionally completely female flowers (apart from 'abnormals' discussed in the next section).

The importance of 'femaleness' to our immediate subject is that the condition of male sterility is often, but not always, correlated with obvious reduction in size of the petals. Such correlation is common in gyno-dioecious species and we have recorded it as a characteristic feature in such genera as *Ranunculus*, *Silene*, *Centaurea*, and *Glechoma* (and other genera of Labiatae). Two examples must suffice for *Saxifraga granulata*:

155/36. Sepals and gynoecium normal in size, shape, and function. Petals well formed but below average size, 8 mm. long, 3·5 mm. broad. Stamens with distinct filaments and anthers, but former short (2 mm. long) and latter small (0·75 mm. long) and without pollen grains.

155/41. Sepals and gynoecium normal. Petals normal and full size, 14 mm. long, 5 mm. broad. Stamens with well-formed filaments (4 mm. long) but anthers small (0·75 mm. long) and without pollen grains.

155/36 is typical for most 'female' flowers; 155/41 is an unusual condition in the population and families studied.

Abnormals. The structural details in abnormal flowers varied greatly from plant to plant and sometimes, but not always, from flower to flower of one plant. In all the numerous abnormal flowers dissected the calyx and gynoecium were normal, except in one sample (117/21) in which the carpels were open at the level of the bases of the styles exposing both loculi. The androecium is most modified but the petals are also frequently more or less changed in structure and texture in abnormal flowers. Generally, the more modified is the androecium the more changed are the petals. Rarely, abnormalities in some or all of the stamens occur in a flower with a normal corolla. No flower with petal modifications of the types described in the immediately following paragraphs has been found without metamorphosed stamens or such as do not produce a full complement of viable pollen.

The following examples will serve to illustrate the abnormalities recorded and together approximately cover the range of these:

119/22. This represents, with minor variations, the commonest abnormality.

Petals smaller than the average, 7–8 mm. long, yellowish green in colour, of rather thick texture, with a tendency to produce a keel; multicellular glandular hairs present similar to those on the sepals, but fewer in number both absolutely and per unit area of surface; venation different from the normal in the anastomosing of at least the three main veins near the apex of the petal. In all these characters the petals are subsepaloid. All ten stamens metamorphosed into subcarpellary structures; the upper part in the mature organ is expanded to a flat stigmatose area covered with stigmatic hairs; below this the

organ becomes hollowed in a boat-like manner, culminating in a fairly deep sac; the impression is of a half-formed sterile carpel with open edges and no ovules; in the young condition the upper part curves over adaxially (strongly cucullate). It is as if the genes responsible for the abnormality have so acted as, metaphorically speaking, to push the petals down towards the sepals and the stamens up towards the carpels.

119/21. Petals rather smaller than usual, flushed with purple and yellowish green, with thicker texture and multicellular glands, but main veins open and not anastomosing. The stamens are normal in appearance but about 50% of the pollen grains are ill-formed or have collapsed.

119/50. Petals as in 119/21. In one flower five stamens opposite sepals normal; five stamens opposite petals subcarpellary and sometimes with an extra substigmatic branch. Other flowers still more abnormal.

117/21. Petals reduced in size and greenish yellow with some multicellular glands, but venation normal. Androecium showing all stages between stamens and subcarpellary organs; some fairly normal anthers have the connective growing out as a reduced stigma and there are all degrees of metamorphosis of the anther to organs with no trace of polliniferous tissue.

121/39. Petals partly lobed and with a tendency to metamorphose into foliage leaves. Androecium with one normal stamen and the remainder more or less petaloid or lobed and intermediate between petals and foliage leaves. Three or four other flowers on the plant were similar in structure while the remainder had petals reduced in size, of thicker texture, and flushed with purple or green or both, but with more or less normal polliniferous stamens.

163/40. Petals rather smaller than the average. Androecium of subpetaloid organs showing varied combinations of characters of petals, stamens, and carpels, and not all alike; one such organ had a flat petaloid expansion with petaloid venation and, on its adaxial surface, two 'enations' right and left of the main vein which was terminated by a reduced stigma; another was subcarpelloid as in 119/22; another showed some attempt to produce polliniferous tissue on one side.

159/29, 163/17, 164/4. Petals normal and well developed. Androecium with some normal or at least polliniferous stamens, but other stamens branched with subpetaloid or subcarpellary branches and a more or less well-formed to abortive anther.

Analysis of petal size and shape in a random sample of fifty plants from near Hog's Back, Surrey (measurements in mm.)

	Maximum	Minimum	Mean	Standard deviation of mean	Standard error of mean
Length	16·0	9·0	13·42	1·785	0·253
Breadth	8·5	3·0	4·93	1·276	0·180
Height above base of widest part	13·0	6·0	9·10	1·775	0·251
Ratio of length to breadth	4·0	1·8	2·83	0·592	0·837
Correlation of length to breadth			$r = 0{\cdot}476$, S.E. of $r = 0{\cdot}1094$		

Petals overlap 22; contiguous 1; divergent 26; uncertain 1; as scored at full anthesis but before recurving of petals.

General outline shape of petals obovate 12; oblanceolate 24; narrowly oblanceolate-oblong 14.

Apex of petals obtuse 18; emarginate 13; rounded 19.

The above figures may be taken as a fair sample of what actually occurs in the wild population. It must, however, be noted that gene segregation and recombination are in a sense limited by vegetative propagation which occurs readily by means of the numerous small bulbs already mentioned. Further investigation is needed to discover the proportion of reproduction by seeds to propagation by bulbs.

ANALYSIS OF PETAL SIZE AND SHAPE IN FAMILIES BRED UNDER CONTROLLED POLLINATION

The stock plants used in the experiments recorded below were all from the population near Hog's Back. They were selfed or crossed (after de-anthering) in an insect-proof breeding house at Potterne and the resulting seeds of all families were sown and the seedlings eventually pricked out and transplanted, under conditions as uniform as possible, into beds out-of-doors on Upper Greensand soil. For analysis of the soil at Potterne reference may be made to *J. Ecol.* **18**, 353–4 (1930).

The twenty individuals grown as stock plants (not all were used in this series of experiments) when analysed as one (not random) sample gave the following results:

Length				
Max.	Min.	Mean	S.D. of mean	S.E. of mean
16·0	8·0	11·8	2·4	0·54
Breadth				
Max.	Min.	Mean	S.D. of mean	S.E. of mean
6·0	3·0	4·3	1·07	0·24
Height above base of widest part				
Max.	Min.	Mean	S.D. of mean	S.E. of mean
12·0	5·0	8·3	2·2	0·49

Table 1 gives maximum, minimum, mean, standard deviation of the mean, and standard error of the mean for families raised as selfings, F_1, F_2, and backcrosses of stock plants, as noted in the column headed 'parentage', for the petal characters length, breadth, and height above base of widest part. Figures for relevant stock plants are appended at the end of the table.

GENETICS OF 'ABNORMAL' FLOWERS

As already described, abnormalities in the androecium are most often, though not always, associated with obvious modifications of the petals. It is, therefore, within the scope of this paper to consider the breeding behaviour of plants showing, or producing descendants showing, such abnormalities. Moreover, it is necessary, for an analysis of the 'normal' quantitative characters for petal size, not to confuse the families derived from or involving 'abnormal' parents with those involving only 'normal' parents.

From the descriptions of abnormal flowers given above it will be realized that the term 'abnormal' covers a wide range of structural modifications. Since many of these may occur in flowers of one plant, and several even in one flower, and normal and abnormal flowers may occur in the same inflorescence, it is impossible to score plants into classes according to the kind or degree of abnormality. In the whole of this section, unless otherwise stated, 'abnormal' refers to abnormalities of the androecium, whether associated or not associated with abnormalities of the petals, and a plant has been scored as

Table 1

Family no.	Parentage	No. of plants in family	Length					Breadth					Height above base of widest part				
			Max.	Min.	Mean	S.D. of mean	S.E. of mean	Max.	Min.	Mean	S.D. of mean	S.E. of mean	Max.	Min.	Mean	S.D. of mean	S.E. of mean
121	S.P. 22 selfed	54	15·0	6·0	9·7	2·38	0·324	8·0	2·0	4·4	1·37	0·186	11·0	3·0	6·1	1·89	0·257
163	121/7 selfed	47	13·0	7·5	10·0	1·24	0·181	7·0	3·5	4·5	0·73	0·106	8·0	3·5	6·3	1·09	0·159
164	121/16 selfed	62	12·0	6·5	9·4	1·39	0·177	6·0	2·5	4·4	0·94	0·120	8·0	3·5	6·1	1·42	0·180
165	121/37 × 121/7	27	11 0	7·0	8·8	1·08	0·208	5·0	2·5	3·5	0·61	0·117	8·0	4·0	5·2	0·96	0·185
127	S.P. 22 × S.P. 6	23	14·0	11·5	12·4	0·68	0·142	7·0	5·0	5·9	0·62	0·129	11·0	8·0	8·7	0·78	0·163
119	S.P. 23 selfed	60	10·0	6·0	8·2	1·26	0·163	4·5	2·0	3·2	0·60	0·085	7·5	3·5	4·9	0·82	0·105
159	119/19 × 119/23	27	13·0	7·5	9·9	1·37	0·263	5·5	3·0	4·0	0·75	0·144	10·0	4·5	6·3	1·20	0·231
161	119/23 selfed	44	12·0	7·5	10·0	0·99	0·149	6·5	4·0	4·9	0·33	0·050	8·0	4·5	6·5	0·89	0·134
162	119/29 selfed	51	13·5	7·0	9·8	1·46	0·204	5·5	2·5	4·0	0·71	0·100	8·0	4·0	6·0	1·03	0·144
160	119/19 × 123/52	53	15·0	10·0	12·8	1·03	0·141	7·0	3·5	5·5	0·72	0·099	11·0	5·5	8·3	1·19	0·163
117	S.P. 24 selfed	29	10·0	6·0	8·5	1·14	0·212	4·0	2·5	3·4	0·49	0·091	7·0	3·0	5·0	1·05	0·195
156	117/10 selfed	15	9·5	5·0	7·4	1·09	0·282	5·0	2·5	3·7	0·66	0·171	6·0	3·0	4·7	0·77	0·199
157	117/14 selfed	50	13·0	8·5	10·4	1·16	0·164	6·5	4·0	4·9	0·56	0·079	9·0	5·0	6·8	0·94	0·133
158	117/22 selfed	41	13·5	7·0	9·8	1·32	0·206	5·0	2·5	4·0	0·67	0·102	7·5	4·0	5·7	0·98	0·153
114	S.P. 24 × S.P. 29	39	16·5	7·5	12·4	1·87	0·300	6·0	2·5	3·9	0·95	0·152	12·0	4·5	7·9	1·56	0·250
115	S.P. 24 × S.P. 22	38	12·0	5·5	8·0	1·56	0·253	5·5	2·0	3·0	0·73	0·119	8·0	3·0	4·9	1·09	0·177
110	S.P. 9 × S.P. 24	48	14·0	10·5	12·3	0·94	0·136	8·0	4·5	5·6	0·59	0·085	10·0	6·5	8·1	0·91	0·131
152	110/2 selfed	34	16·0	12·0	13·6	0·93	0·159	9·0	6·0	7·5	0·89	0·153	12·0	8·0	9·3	0·80	0·137
153	110/24 selfed	28	16·0	12·0	14·0	0·93	0·176	8·5	5·0	6·6	0·97	0·183	12·0	8·0	9·8	0·88	0·166
126	S.P. 6 selfed	28	13·0	10·0	11·5	0·89	0·169	8·0	5·0	6·4	0·77	0·146	10·0	6·0	8·2	0·83	0·157
124	S.P. 29 selfed	30	17·0	12·0	14·8	1·12	0·204	4·0	2·5	3·0	0·09	0·017	14·0	8·0	11·2	1·58	0·288
170	124/23 selfed	56	17·5	11·0	15·2	1·29	0·172	5·5	3·0	3·9	0·61	0·082	13·0	8·0	10·1	0·93	0·124
123	S.P. 6 × S.P. 29	54	15·0	11·0	13·0	1·13	0·154	6·5	3·5	5·0	0·74	0·101	11·0	7·0	9·2	1·07	0·146
167	123/8 selfed	67	17·0	10·5	13·5	1·42	0·173	9·0	4·0	6·3	0·71	0·087	12·0	7·0	9·3	1·17	0·143
168	123/36 selfed	10	20·0	14·0	15·8	1·64	0·519	5·5	4·0	4·6	0·49	0·155	17·0	9·5	11·0	2·09	0·661
169	123/52 selfed	75	15·5	10·5	12·8	1·14	0·131	7·5	4·0	5·9	0·82	0·095	10·0	6·0	8·3	1·12	0·129
166	123/5 × 123/8	50	17·0	11·0	13·8	1·46	0·207	7·0	3·5	5·3	0·78	0·110	13·0	7·0	9·8	1·34	0·190
116	S.P. 29 × S.P. 6	32	16·5	12·0	13·9	1·26	0·223	8·0	4·0	5·5	0·78	0·138	12·0	8·5	9·8	0·85	0·168
154	116/19 selfed	55	17·5	11·5	14·3	1·50	0·202	7·0	3·5	5·4	0·73	0·098	14·0	8·0	9·9	1·33	0·179
155	116/23 selfed	54	16·0	10·0	12·6	1·34	0·182	7·0	4·0	4·8	0·75	0·102	11·0	7·0	9·0	1·04	0·141
128	S.P. 14 × S.P. 6	55	15·0	10·0	12·2	1·22	0·165	8·5	4·0	6·0	0·90	0·121	11·5	7·0	8·9	0·32	0·043
131	S.P. 9 selfed	23	14·0	11·0	12·0	0·71	0·148	7·0	5·0	5·7	0·49	0·102	10·0	8·0	8·7	0·69	0·144
118	S.P. 27 selfed	20	18·0	11·0	14·5	1·55	0·347	5·5	3·0	4·0	0·55	0·123	12·5	8·0	10·2	1·11	0·246
130	S.P. 27 × S.P. 9	29	16·0	11·0	13·5	1·19	0·223	6·5	3·5	4·8	0·75	0·139	12·0	8·0	9·4	1·26	0·234
109	S.P. 9 × S.P. 27	64	17·5	11·0	14·0	1·42	0·177	8·0	4·0	5·2	0·79	0·099	14·0	7·0	9·8	1·21	0·151
148	109/28 selfed	37	16·5	10·0	13·8	1·45	0·238	8·0	4·0	6·4	1·05	0·173	12·0	8·0	9·5	1·07	0·176
149	109/29 selfed	70	19·0	10·5	15·2	1·88	0·225	8·0	4·5	5·9	0·86	0·103	14·0	8·0	10·8	1·48	0·177
150	109/38 selfed	62	19·5	13·5	16·1	1·33	0·169	7·5	3·5	5·3	0·82	0·104	14·5	9·0	11·5	1·20	0·152
151	109/60 selfed	66	19·0	13·0	15·0	1·09	0·134	7·0	4·0	5·7	0·70	0·086	14·0	8·0	10·5	1·17	0·144
112	S.P. 27 × S.P. 29	19	17·0	13·5	15·2	0·97	0·222	5·0	3·0	4·0	0·51	0·117	12·0	9·0	10·3	0·76	0·174
108	S.P. 15 selfed	13	12·5	9·0	10·1	1·20	0·332	5·0	3·5	3·9	0·46	0·127	9·0	4·5	6·9	1·12	0·310
120	S.P. 18 selfed	61	19·0	11·0	15·1	1·79	0·229	8·0	4·5	5·7	0·94	0·120	13·5	8·0	10·5	1·43	0·183
	S.P. 6	—	11·5	—	11·0	—	—	—	—	6·0	—	—	—	—	8·5	—	—
	S.P. 9	—	—	—	12·0	—	—	—	5·0	6·0	—	—	—	—	9·0	—	—
	S.P. 14	—	11·0	8·0	9·5	—	—	5·5	3·5	4·5	—	—	—	—	6·5	—	—
	S.P. 15	—	—	11·0	11·5	—	—	—	3·0	4·5	—	—	—	—	8·0	—	—
	S.P. 18	—	—	—	14·0	—	—	—	—	4·5	—	—	—	—	10·0	—	—
	S.P. 22	—	—	9·0	10·0	—	—	3·5	2·5	3·0	—	—	—	—	6·0	—	—
	S.P. 23	—	—	—	9·0	—	—	—	—	3·0	—	—	7·0	—	5·0	—	—
	S.P. 24	—	—	—	8·0	—	—	—	—	3·0	—	—	—	5·0	5·5	—	—
	S.P. 27	—	—	—	16·0	—	—	—	—	3·5	—	—	—	—	12·0	—	—
	S.P. 29	—	—	14·5	15·0	—	—	—	—	3·5	—	—	—	—	12·0	—	—

'abnormal' if in any flower abnormal stamens have been found. It is possible that a few plants have been scored as normal though they are genetically low abnormals, because a low degree of abnormality is sometimes expressed only in late developing flowers, and in plants of poor growth, these may never reach full anthesis or may not have done so at the times of scoring. In order to reduce differential environmental effects on the quantitative characters being studied it was necessary to collect the material for scoring within a very limited period, and nearly all of it was collected and pressed within two days.

A preliminary survey of the figures for 'abnormals' showed that in their production genes from three stock plants were involved: S.P. 22, S.P. 23 and S.P. 24. The three stock plants themselves showed a very low degree of abnormality with only some stamens in a few flowers affected. It may be noted that plants with a high degree of androecial abnormality cannot be selfed or used as pollen parents but, at most, only as ovule parents. The results obtained by utilizing the above three stock plants may be arranged as follows:

S.P. 22

Family no.		
121	(=S.P. 22 selfed)	gave 36 normals : 18 abnormals
163	(=121/7 selfed)	gave 24 normals : 23 abnormals
164	(=121/16 selfed)	gave 18 normals : 44 abnormals
165	(=121/37 × 121/7)	gave 0 normals : 25 abnormals
127	(=S.P. 22 × S.P. 6)	gave 23 normals : 0 abnormals

S.P. 23

119	(=S.P. 23 selfed)	gave 27 normals : 33 abnormals
159	(=119/19 × 119/23)	gave 8 normals : 19 abnormals
161	(=119/23 selfed)	gave 42 normals : 2 abnormals
162	(=119/29 selfed)	gave 40 normals : 11 abnormals
160	(=119/19 × 123/52)	gave 51 normals : 2 abnormals

S.P. 24

117	(=S.P. 24 selfed)	gave 10 normals : 18 abnormals
156	(=117/10 selfed)	gave 0 normals : 15 abnormals
157	(=117/14 selfed)	gave 46 normals : 4 abnormals
158	(=117/22 selfed)	gave 7 normals : 34 abnormals
114	(=S.P. 24 × S.P. 29)	gave 28 normals : 11 abnormals
115	(=S.P. 24 × S.P. 22)	gave 6 normals : 32 abnormals
110	(=S.P. 9 × S.P. 24)	gave 48 normals : 0 abnormals
152	(=110/2 selfed)	gave 34 normals : 0 abnormals
153	(=110/24 selfed)	gave 28 normals : 0 abnormals

It is obvious that no simple monohybrid or dihybrid ratio will explain the above figures but that a considerable number of interacting genes are involved. The following results are instructive:

(1) The selfing of the three stock plants gave considerable numbers of 'abnormals', considering the very low degree of abnormality shown by the parents, with ratios of normals to abnormals of: 2 : 1; 9 : 11; and 5 : 9 respectively.

(2) Crossing of one of these stock plants with another stock plant with no factors for abnormality (so far as shown by our experiments) gave families with no or very few abnormal plants (families 127, 160, and 110) and two F_2 families from such a cross (namely 152 and 153) gave no abnormal plants. An exception to this rule was given by 114, but this is in agreement with other figures showing that S.P. 24 carried a relatively high number of factors for abnormality even though it had itself a low expression of abnormality. It should be noted that in family 127 the normal plant was the pollen parent and in family 110 it was the ovule parent, but in each family there were no abnormals.

(3) Crossing of two (slightly) abnormal stock plants (S.P. 24 × S.P. 22) gave a family (115) with a very high proportion of abnormals (ratio of 1 normal : 5·3 abnormals).

(4) Considerable and variable segregation of the factors involved in normality and abnormality is shown by the families derived by selfing individuals from families themselves derived from selfing a stock plant (F_2 of selfings). These involve the following (exact or very approximate) ratios of normals to abnormals: 1 : 1, 1 : 2, 22 : 1, 4 : 1, 0 : 15, 11 : 1, and 1 : 5.

(5) Crossing of two sibs of a family produced by selfing a stock plant has twice (families 165 and 159) resulted in relatively high numbers of abnormals with the ratios of normals to abnormals 0 : 25 and 8 : 19 respectively.

It has already been stated that in the majority of abnormal flowers both androecium and corolla are affected. In general, this is well marked when there is a high degree of abnormality and in families with a high proportion of abnormals. Family 115 is in particular a good example of this and of the marked petal contrast between flowers with normal and those with abnormal androecium. There are, however, important exceptions to this generalization. For example, family 121 had, for androecium, 36 normals : 18 abnormals. Taking petals also into account the following figures were obtained: 21 petals and stamens normal : 15 petals abnormal, stamens normal : 0 petals normal, stamens abnormal : 18 petals abnormal, stamens abnormal. In other families plants (or flowers) with normal petals and abnormal stamens occur. It is clear that a number of interacting factors are concerned in producing normality or abnormalities (of the kinds here examined) of the corolla and androecium but that they (at least in their different combinations) are not all of the same value and do not have simple additive effects. Some factors for abnormality can be and apparently are recessive to the normal singly or in combinations but others are probably partially expressed in the heterozygous condition.

We have now to consider the influence of these abnormalities on the statistics of petal size. It must be recalled: first, that most of the families with abnormal plants contain also a varying number of normal plants with fully developed petals; secondly, that there are all degrees of petal abnormalities varying from plant to plant and even from flower to flower of one plant; and, thirdly, that the best developed (least abnormal) petals were taken for measurements in abnormal plants.

The following figures show that even with the above limitations there are considerable differences between families for average petal size due to the occurrence or non-occurrence in a family of abnormal plants. Had abnormal petals been selected for measurement the differences would have been much greater.

28 families involving only normals		13 families derived from stock plants 22, 23, and 24 only		6 families derived from abnormal stock plants crossed with normals	
Average mean length	Average mean breadth	Average mean length	Average mean breadth	Average mean length	Average mean breadth
13·8	5·2	9·2	3·9	12·9	5·9

These figures may also be compared with the means for the random sample of the wild population of length 13·4 and of breadth 4·9; and for those of the stock plants of length 11·8 and of breadth 4·3.

The high average mean for breadth in the families derived from abnormal stock plants crossed with normals is, in part, due to the absence of plants with long narrow strap-shaped petals as compared with their presence in some of the families involving only normals.

ANALYSIS OF PETAL SIZE AND SHAPE IN FAMILIES FROM NORMAL STOCK PLANTS

Of the three measurements taken, that of height above base of widest part is, for reasons already given, the least satisfactory. While it did not appear, from preliminary tests, worth while working out in detail the correlations of this character with length and breadth of petals the measurements taken from all petals show that the widest part or the centre of the widest part is always above the middle of the petal. Thus, in the terminology of descriptive botany, the petals are obovate, oblanceolate, or narrowly oblanceolate-oblong. It is length and breadth which determine the main differences in size and shape while position of the widest part fluctuates only in the upper half of the petal and mostly in the lower or middle third of the upper half. It is with length and breadth and especially the ratio between them that the remainder of this section is concerned.

The stock plants showed the following ratios of length to breadth:

Stock plant	L/B	Stock plant	L/B	Stock plant	L/B	Stock plant	L/B
2	2·40	9	2·00	17	3·00	23	3·00
4	2·89	10	2·25	18	3·11	24	2·67
5	1·82	13	4·00	19	2·00	25	2·75
6	1·83	14	2·11	21	2·89	27	3·89
8	2·86	15	2·56	22	2·86	29	4·29

Families involving stock plant 6

Stock plant 6 had petals rather below average length but above average breadth. It was selfed and was crossed reciprocally with stock plant 29 (with long, narrow petals), with stock plant 14 (with short, approximately medium width petals), and with stock plant 22 (slightly abnormal and giving a proportion of abnormal offspring). The last cross has already been considered.

Taking the ratio length to breadth and grouping into classes with intervals of 0·2 (i.e. from 1·26 to 1·45, 1·46 to 1·65, etc.) the results shown in Table 2 are obtained, the classes being designated by the odd whole number after multiplication by 10 of the actual ratios.

Table 2

Family no.	13	15	17	19	21	23	25	27	29	31	33	35	37	39	41	43	45	47	49	51	53	55	57	59	61	63	Mean
126	1	10	7	6	3	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1·80
124	—	—	—	—	—	—	—	—	—	—	1	0	0	1	1	0	3	6	7	3	4	1	2	0	0	1	4·93
170	—	—	—	—	—	1	0	1	3	3	3	3	6	10	4	9	4	2	6	0	1	—	—	—	—	—	4·00
123	—	—	—	2	7	15	7	9	8	0	4	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2·62
167	—	2	6	21	16	4	15	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2·17
168	—	—	—	—	—	—	—	—	2	2	3	1	0	1	0	0	0	0	1	—	—	—	—	—	—	—	3·48
169	—	3	4	15	25	14	8	3	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2·34
166	—	—	—	—	10	6	9	10	7	4	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2·65
116	—	—	—	3	1	9	8	7	1	2	0	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2·55
154	—	—	—	1	5	13	13	7	9	3	3	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2·63
155	—	—	—	2	10	4	9	12	13	2	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2·65
128	2	3	11	10	13	13	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2·05

On the whole these figures are very close to what might be expected, as the following comments indicate:

(1) Stock plant 6 (in L/B class 17) on selfing gave family 126 with a mode in class 15 but a mean of 1·80, i.e. in class 17.

(2) Stock plant 29 (in L/B class 43) on selfing gave family 124 with a mode in class 49 and a mean of 4·93. The considerable differences with disjunction of classes in the families derived from selfing stock plants 6 and 29 are important.

(3) A selfing of 124/23 gave family 170 with a mode in class 39 and a mean of 4·00.

(4) The F_1 from stock plant 6 × stock plant 29 (family 123) has a mode in class 23 and a mean of 2·62.

(5) F_2 families from this cross (families 167, 168, and 169) cannot be equated and obviously represent offspring from immediate parents of different factorial content for petal characters. The class frequencies appear, indeed, to be related to the L/B ratios of the immediate parents: 123/8 (parent of family 167) with L/B = 2·18; 123/36 (parent of family 168) with L/B = 3·0; and 123/52 (parent of family 169) with L/B = 2·40.

(6) Family 166 resulted from the crossing together of two F_1 sibs. It is bimodal (classes 21 and 27) and has a mean of 2·65.

(7) Family 116 resulted from the reciprocal cross of family 123. Its mode is in class 23 and its mean is 2·55, agreeing well with family 123.

(8) The two F_2 families (154 and 155) have modes at 23–25 and 29 and means of 2·63 and 2·65 respectively.

One other family involves stock plant 6, namely family 128 (S.P. 14 × S.P. 6). Stock plant 14 had L/B ratio 2·11 and in stock plant 6 L/B = 1·83. Family 128 has the mode 21–23 and the mean 2·05.

Families involving stock plant 9

Stock plant 9 had petals very near to the mean length for the stock plants but was maximum in breadth, giving the L/B ratio of 2·00. It was selfed and was crossed reciprocally with stock plant 27 (with long narrow petals) and with stock plant 24 (slightly abnormal and giving a proportion of abnormal offspring on selfing). The last F_1 and its F_2 have already been considered. Utilizing the same scheme as for families involving stock plant 6, the results shown in Table 3 are obtained.

Table 3

Family no.	15	17	19	21	23	25	27	29	31	33	35	37	39	41	43	45	47	Mean
131	—	2	5	14	1	0	1	—	—	—	—	—	—	—	—	—	—	2·11
118	—	—	—	—	—	—	1	1	1	5	4	3	1	0	2	1	1	3·63
130	—	—	1	1	1	9	5	3	5	2	1	0	1	—	—	—	—	2·87
109	—	—	1	7	13	14	6	10	5	4	2	1	1	—	—	—	—	2·71
148	1	6	10	3	13	1	0	3	—	—	—	—	—	—	—	—	—	2·18
149	—	—	2	3	19	18	15	7	2	3	0	0	0	1	—	—	—	2·65
150	—	—	—	2	3	6	7	16	5	11	6	1	2	2	1	—	—	3·10
151	—	—	—	7	12	13	18	9	4	2	1	—	—	—	—	—	—	2·67

Again, these figures clearly suggest a genetic basis for the L/B ratio, as the following comments indicate:

(1) Stock plant 9 (with L/B 2·00) on selfing gave family 131 with a mode in class 21 and a mean of 2·11.

(2) Stock plant 27 (with L/B 4·57) on selfing gave family 118 with a mode in class 33 and a mean of 3·63.

(3) Stock plant 27 × stock plant 9 gave an F_1 (family 130) with a mode in class 25 and a mean of 2·87, while the reciprocal (family 109) gave a mode in the same class and a mean of 2·71.

(4) The F_2 families (148, 149, 150, 151) from the cross stock plant 9 × stock plant 27 gave results which cannot be equated one with another and represent offspring from immediate parents of different gene content for petal characters. The class frequencies show some connexion with the L/B ratios of the immediate parents, but not so clearly as in F_2

families from the crossing of stock plant 6 and stock plant 29. This may be due to the occurrence of femaleness in a number of the F_2 plants (particularly in family 148). Thus: 109/28 (parent of family 148) had L/B = 2·60; 109/29 (parent of family 149) had L/B = 2·50; 109/38 (parent of family 150) had L/B = 3·40; and 109/60 (parent of family 151) had L/B = 2·38.

Families involving various stock plants

One cross and two selfings remain for consideration. For these the tabulated results are:

Family no.	19	21	23	25	27	29	31	33	35	37	39	41	43	45	47	49	Mean
112	—	—	—	—	—	—	3	3	4	1	2	2	1	0	2	1	3·84
103	1	2	0	6	0	3	1	—	—	—	—	—	—	—	—	—	2·58
120	3	4	11	10	17	12	3	1	—	—	—	—	—	—	—	—	2·66

The following comments may be made:

(1) Family 112 was obtained by crossing together stock plant 27 and stock plant 29, both with long narrow petals with the L/B ratios = 4·57 and 4·29 respectively. With mode in class 35 and mean of 3·84 there was a reduction in the value of the L/B ratio, which, however, is still high. The family was a small one of 19 plants.

(2) Family 103 was obtained by the selfing of stock plant 15 with L/B ratio = 2·56. The mode for the family was in class 25 and the mean was 2·58.

(3) Family 120 was obtained by the selfing of stock plant 18 with L/B ratio = 3·11. The mode was in class 27 and the mean was 2·66.

CONCLUSIONS

The statistical analysis of petal size and shape in *Saxifraga granulata* recorded in this paper shows that the genetic basis is complicated. It is clear that chromosomal genes are responsible for the general shape and there is no suggestion of cytoplasmic influences since reciprocal crosses give very similar results. Environment conditions, including position of flowers in the inflorescence, can modify absolute size of petals in common with that of other floral parts, but appear to have little or no influence on the L/B ratio though this latter varies with age of the flowers. The widest portion is always above the middle and fluctuates mainly within the lower two thirds of the upper half. It has not been possible to prove any genetical basis for variations in the position of the widest portion of the petals and there may be none beyond those which keep it constantly in the upper half. In regard to size and shape as determined by greatest length and greatest breadth and the ratio between them there are at least three groups of factors involved:

(*a*) A basic set which determines size and shape in general terms as seen in normal hermaphrodite flowers.

(*b*) A genom with factor or factors determining femaleness and resulting in somewhat smaller petals than when the genom, otherwise the same, results in or allows the development of normal hermaphrodite flowers.

(*c*) A set of factors resulting in 'abnormalities' of the androecium and/or the petals.

'Female' flowers (with contabescent anthers and the stamens more or less reduced in size and without viable pollen but not otherwise abnormally developed) are not sufficiently frequent in the material studied to affect the statistical results to a very appreciable extent. The 'basic' set and the 'abnormal' set of factors each involve a considerable number of segregating genes to the extent that variations in size, shape, and abnormalities

can be so arranged as to be essentially continuous and any scoring into classes becomes arbitrary.

Different chromosome numbers have been recorded by several cytologists for *S. granulata*. It is sufficient here to note that Philp (*J. Genet.* **29**, 197–201, 1934) found 32 chromosomes and concluded from his observations that the species was hexaploid, and the fact that bivalent formation is the rule indicates that it is an allopolyploid. On this basis alone one might expect multiple genes for petal size and shape. It has not, however, been possible from the data available at present to determine the number of gene loci involved, but there may well be at least a dozen in the 'basic' and 'abnormal' sets together.

Our thanks are due to Miss F. Hamilton for assistance in measuring petals of bred families, to H. Montford, Esq., B.Sc., for assistance in investigating the wild population, and to Mrs F. E. Turrill for help with the statistical analysis. The research on which this paper is based has been aided by a Royal Society Government Grant.

EXPLANATION OF PLATES 8–10

Plate 8

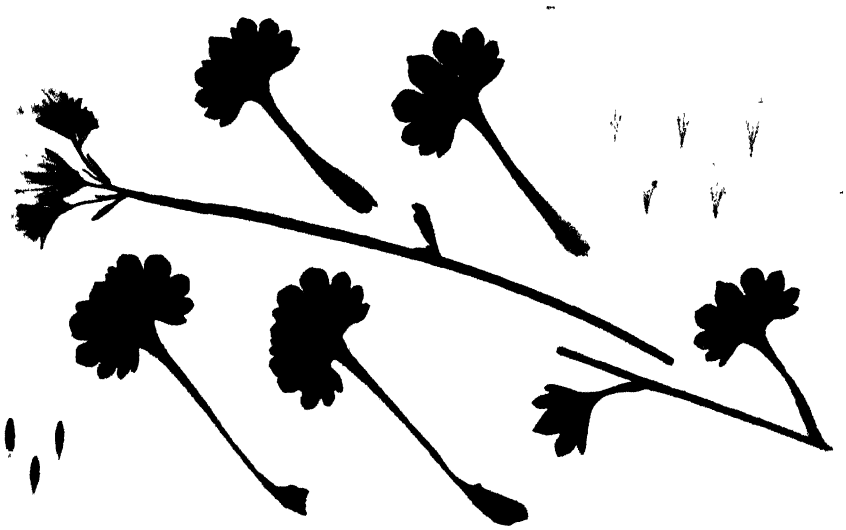
Saxifraga granulata. Stock Plant 15, near Hog's Back, Surrey, 1930, on Folkestone Sands. *Saxifraga granulata.* Stock Plant 9, near Hog's Back, Surrey, 1930, on Folkestone Sands.

Plate 9

Saxifraga granulata. Petals of stock plants, from near Hog's Back, Guildford district, Surrey, 1930, on Folkestone Sands.

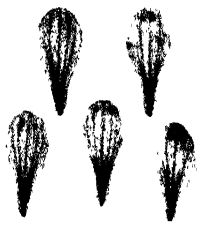
Plate 10

Saxifraga granulata. Petals of stock plants, from near Hog's Back, Guildford district, Surrey, 1930, on Folkestone Sands.



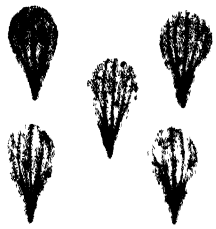
S P. 15

S P. 9



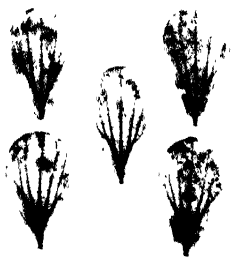
S.P. 2

S.P. 4

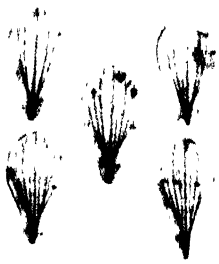


S.P. 5

S P 6



S P. 10

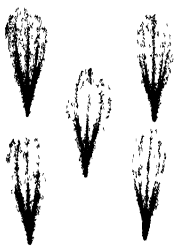


S P 14

S.P 8

S.P. 9

S P. 13



S.P. 15

S.P. 17

S.P. 18

S.P. 21

S.P. 22

S.P. 23

S.P. 24

S.P. 25

S.P. 27

S.P. 29

HYBRIDIZATION IN *TRITURUS*

I. DESCRIPTION OF A METHOD BY WHICH RECIPROCAL HYBRIDS FROM *TRITURUS TAENIATUS* AND *TRITURUS ALPESTRIS* CAN BE OBTAINED

By H. E. BRAAKSMA

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The purpose of this investigation is the study of chromosomes in *Triturus taeniatus*, *T. alpestris* and in their reciprocal hybrids. In this first short paper a method will be described by which reciprocal hybrids between both *Triturus* species can be obtained. Spontaneous mating between specimens of different species of *Triturus* generally does not occur and certainly not so between *T. taeniatus* and *T. alpestris*. Many investigators, including Born (1884), Gebhardt (1894), Steiner (1927), Bataillon (1927–30), Baltzer (1934), Hamburger (1935, 1936), have tried hybridization between different amphibian species and different genera before with more or less success. Most of these investigators worked by the following method: Eggs from the ovary of a killed female specimen were placed in a physiological salt solution, and sperm of a male specimen of a different species or even genus was added. This method has the drawback that many females must be sacrificed, as only a small part of the eggs present in the tubes and ovaries are mature. Hence the other eggs are all lost. Moreover, the chance for fertilization in a physiological salt solution is less than normal, while the eggs are damaged more easily. In using this method many hybrids die at the gastrula stage, which shows their inferiority; a number of them, however, become full grown (Gebhardt, 1894; Hamburger, 1935, 1936). It is possible that these losses are caused by the unnatural way of fertilization. The drawbacks, mentioned above, can simply be avoided by fertilizing the eggs under more physiological circumstances, namely, in the cloaca of the female (as happens in nature, according to Zeller). It must be mentioned, however, that, according to Spallanzani, it is possible that in nature fertilization also occurs in the oviduct.

With the following new method one can obtain good results in crossing *T. taeniatus* and *T. alpestris*. These Urodeles mate externally, while fertilization is internal. During the period of mating the male, after a preliminary phase, places one spermatophore in front of the female. This spermatophore consists of a massive gelatinous cone with a broad base, while on its top a little white cap with spermatozoa is situated. Microscopically the cone shows a pearl-rope construction. Beads of jelly run from the base upward and join at the top under the cap containing the spermatozoa. The whole cone is about 1 cm. in height; the midline of the base has the same length. The female, which continuously follows the male, picks up this spermatophore between the cloacal lips. If one siphons off the spermatophore immediately after its deposition and transfers it into the cloaca of a female of a different species, we have found that hybrids will develop from her eggs, which in this way have been fertilized.

According to Kingsbury & Zeller, it might be possible that still living spermatozoa of a former copulation period, consequently even from 1 or 2 years ago, should be present in the spermathecae of the female. In *Triturus* these spermathecae, little blind receptacles,

are to be found in the wall of the cloaca. Each side contains eight to fifteen spermathecae. So one has to be careful that the females, which are to be artificially inseminated, are separated from the males for a long time, or that self-reared, and thus controllable and separately bred specimens are used, which have never mated before. It is practically impossible to wash out the cloacae of recently captured specimens, in order to destroy or to remove spermatozoa which are possibly present. One has to be very careful if animals are used, which are captured very early in spring before the mating period. It can be assumed that there had been no spermatozoa in the spermathecae before, if the females, which have been kept separate, produce unfertilized eggs, while, on the other hand, all the eggs are fertilized if spermatozoa are added artificially. If, on the contrary, there had been sperm in the spermathecae, the first laying of eggs would also have been fertilized. According to Zeller, however, living spermatozoa do not survive for a year or longer.

Three females (two *alpestris* and one *taeniatus*) produced only unfertilized eggs, each of them 15–20. Spermatozoa of the other species were placed in the cloaca of these females, and after 1 day these females produced eggs which developed very well. The youngsters are now 6 months old. It must be supposed that either these youngsters are real hybrids or that the spermatozoa have only 'activated' the eggs, which are themselves haploid (or diploid) containing only the protoplasm and chromosome material of the female.

We examined microscopically the cloaca of some of the females which had not yet mated during the year in which the investigation took place. No spermatozoa were found. Certainly, however, it is preferable to use specimens which are bred under control, because in this case the spermathecae are certainly empty before artificial fertilization takes place.

Procedure

Early in spring, in the beginning of March, in some small well-planted aquaria A–F, the following animals are housed:

In A 5–10 *T. taeniatus* females
B 5–10 *T. taeniatus* males
C 5–10 *T. alpestris* females
D 5–10 *T. alpestris* males
E 5–10 *T. taeniatus* females
F 5–10 *T. alpestris* females

One must be careful that the animals in E and F have no spermatozoa in the cloaca or spermathecae, and that they are unfertilized in any way. By using the method mentioned above, this state can be obtained. All animals are copiously fed. After about 10 days, the sex urge is so increased that even females as well as males display for one another. Now in a rather spacious aquarium, with an even bottom and a few plants, some animals from the aquaria A and B are brought together in a proportion of about two males to three females. Mating occurs after some minutes, and the spermatophores are siphoned off and collected, whereafter the animals are brought back to A or B respectively. Used and non-used animals in the same aquarium eventually can be separated by a plate of glass, so that the same animals are not repeatedly used by accident. In this way many spermatophores are soon collected, while at the same time one does not waste much time in waiting for mating, which is always necessary if animals of both sexes are con-

tinually together. Now a spermatophore coming from the males of *T. taeniatus* in the way just mentioned, is brought into the cloaca of each of the females of *T. alpestris* from aquarium F. The simplest way of performing this is by wrapping up the females in some dry cotton-wool, so that the cloacal opening remains free. The fine fibres of the cotton-wool wrap themselves around the legs and the tail of the female as the result of her vigorous movements. In this way the animal soon becomes motionless. The female, thus immobilized, is taken in the left hand with its ventral surface upwards. With the right hand the cloacal opening and its surroundings are wetted with a little syringe. Now also with the right hand one takes the base of the spermatophore by means of tweezers. Its cone is put between the cloacal lips with its point, on which the sperm cap lies, turned downward. The spermatophore may be pushed inwards by means of a thick glass rod, although generally this is not necessary, the adhesive power being strong enough to fix the spermatophore to the cloacal lips. Now the female is separated in a little basin with floating vegetation, which is repeatedly searched for eggs. The same is done with females out of aquarium E which are supplied with spermatophores obtained by mating newts out of the aquaria C and D. The females out of A and C are exclusively used to induce the males of corresponding aquaria to produce spermatophores. During the whole spring the hybridization can be repeated *ad libitum* with the same specimens, depending on the number of hybrids wanted.

Besides its speed and simplicity, this method has the advantage that no animals are sacrificed, the method of fertilization being quite similar to the natural one. Many eggs are supplied with a spermatozoon, and no immature eggs are lost or damaged, which is the case with the method used by the investigators mentioned before. Nearly all the young hybrids—in the same proportion as in normal animals—pass the larval stage and grow up well. This is especially important, because hybrids are very apt to die in the stages of gastrulation, neurulation and metamorphosis. In our method, nature has been imitated as much as possible.

All the hybrid eggs which are produced by a female *T. taeniatus* and a male *T. alpestris* are collected in a spacious aquarium with rich vegetation. The same is done with the reciprocal hybrid eggs. The young animals are fed with *Daphnia*. It is of great importance to clean the bottom of the aquarium regularly.

After metamorphosis the animals are put in a terrarium, in which *Enchytraea* and some slices of bread soaked in milk, are placed some time before and are renewed from time to time. Under these slices a large number of *Enchytraea* develop, on which the young hybrids feed. This method of feeding saves much time.

Since the hybrids obtained by this method are only 6 months old now, their description will be given in a later paper, together with an account of their chromosomes.

SUMMARY

A new method of hybridization in urodeles is described, which gives a maximally good result without sacrificing any animal which is used.

REFERENCES

BALTZER, F. (1934). Ueber die Entwicklung von Triton-Bastarden ohne Eikern. (35. *Jahresvers. d. Dtsch. Zool. Ges.*, Koln, Sitzg. v. 6–8. vi. 1933.) *Zool. Anz.* Suppl. **6**.

BALTZER, F. (1938). Die Frühentwicklung des Bastards *Triton cristatus* × *Triton palmatus*. *Rev. Suisse Zool.* **45**.

BATAILLON, E. (1927). Les croisements chez les urodeles et l'androgénèse hybrides. *C.R. Soc. Biol., Paris*, **97**.

BORN, G. (1884). Ueber die inneren Vorgänge bei der Bastardbefruchtung der Froscheier. *Breslauer ärztlich. Z.* no. 16.

GEBHARDT, W. (1894). Ueber die Bastardierung von *Rana esculanta* und *Rana arvalis*. Inaug. Diss. Breslau.

HAMBURGER, V. (1935). Malformation of hindlimbs in species hybrids of *Triton taeniatus* (and *palmatus*) female × *Triton cristatus* male. *J. Exp. Zool.* **70**.

HAMBURGER, V. (1936). The larval development of reciprocal species hybrids of *Triton taeniatus* Leyd. (and *Triton palmatus*, Dugés) × *Triton cristatus* Laur. *J. Exp. Zool.* **73**.

HERTWIG, G. (1930). Kern- und Zellgröszenunterschiede der Eltern als Ursache des verschiedenen Ausfalls reziproker Krötenkreuzungen. *Z. Anat.* **92**.

MOORE, J. A. (1941). Developmental rate of hybrid frogs. *J. Exp. Zool.* **86**.

ROSTAND, J. (1930). Sur l'hybridation entre *Bufo vulgaris* et *Bufo aqua*. *C.R. Soc. Biol., Paris*, **104**.

ROSTAND, J. (1939). Action du froid sur le développement de l'œuf chez les Batraciens. *C.R. Acad. Sci., Paris*, **209**, 24.

STEINER, H. (1927). Neuere Beobachtungen über *Triton Blassii* de l'Isle. Nochmalige erfolgreiche Kreuzung des *Triton marmoratus* mit *Triton cristatus* subspec. carnifex. *Blatter f. Aquarium- u. Terrariumkunde*, Jg **37**.

SCHOU-SU (1931). Étude cytologique sur l'hybridation chez les anures. *Arch. Anat. micr.* **27**.

OBSERVED SPONTANEOUS MUTATION RATES IN RELATION TO EXPERIMENTAL TECHNIQUE

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Introduction

Mutation rates in the germ cells of organisms which have not been subjected to any form of gross physical or chemical treatment are of special interest to the evolutionary geneticist, since it is probable that they approximate fairly closely to those obtaining under natural conditions. Even though the amount of interference with biological processes and the handling of material associated with laboratory techniques must introduce environmental differences of unknown potency, yet the abundance of existing data obtained on these lines seems to show that this 'laboratory factor' in itself is not sufficiently important to minimize unduly the value of such methods.

The experiments described in this paper were undertaken with the object of measuring mutation frequencies in single individuals of *Drosophila melanogaster* rather than in populations as a whole. It was hoped by this individual sampling method to detect and avoid the inclusion of atypical genetic variants (such as the 'mutator gene' of Mampell (1945) for example) which might distort the results. This was especially important in view of the fact that it was desired in particular to compare the mutation rates in 'marked' and unmarked (wild type) stocks, since the results of previous experiments had appeared to point to some fundamental difference based on the presence or absence of marker genes. (It may at once be said here that the present experiments do not provide conclusive evidence on this point, as problems connected with method supervened and had to be investigated first.) It seemed evident also that the observations must be carried on throughout the whole lifetime of the individual flies in order to examine the possibility of a change in mutation rate in relation to age and similar factors. It would not have been sufficient to study different age groups in different individuals since complementary differences in individuals would tend to mask the facts. The four experiments described below are necessarily of a preliminary nature, since the technique was subject to improvement as the work went on and the method varied in important details. The data obtained, however, appear to be sufficiently interesting to warrant publication and to make further work on the same lines desirable.

General method

Mutation frequencies were measured by the usual method of recording the appearance of new sex-linked lethals in X-chromosomes of sperm, the lethals being detected in the F_2 generation by the non-appearance of males which normally should have contained the grandparental X. However, in view of the low frequencies obtained in untreated material, the definition of lethality has been extended to include a few mutations which did not kill all the males containing it but reduced their numbers sufficiently to give the appearance of full lethals at a preliminary examination.

In all four experiments the offspring of all the P_1 males were recorded and mated individually, and the members of each family and brood were always distinguishable by the use of special markings on the vials.

Males were selected and placed with females not more than 24 hr. after emergence, and were allowed to breed to the end of their life. In general all the F_1 females obtained were mated. Occasional losses through accident occurred, and there was probably some overcrowding in some of the vials, but the great majority of the offspring were saved in most cases. The known exceptions to this rule will be pointed out in the text and tables.

Mutations arising in the females were recorded, but these are not valid for comparison with those arising in the males, since the stocks used were different. The data are therefore not considered in this report.

Experiment A

Thirty-seven virgin females, carrying the sex-linked markers, scute, vermilion, forked, carnation (known as the 'scar' combination and so referred to below) were mated individually to one male each of a scute apricot stock containing inversions sc^8, sc^{s1}, and *In S*, which effectively prevent crossing-over in the *X*-chromosome (with the exception of rare double cross-overs in the cut region, as was later observed). The food used was a modification of Bridge's cornmeal-agar-molasses medium, semolina being used instead of cornmeal, and brown sugar instead of molasses. The vessels used were the usual 1 × 3 in. vials. The flies were kept at a temperature of 24° C. ± 0·5, except when the parents were being transferred to fresh vials, which operation was carried out at room temperature (about 16° C.).

The flies were allowed to lay eggs for 6 days in the first vials. They were then transferred to fresh vials and left another 6 days, after which they were transferred for three more periods of varying length. There were no survivors after the 31st day. There were thus in all five vials, in which each pair were allowed to produce offspring. The F_1 flies emerging in each vial were termed 'broods'. As the numbers in the fifth vials were very small they were added on to those of the fourth vial, making four broods altogether.

Of the thirty-seven pairs twenty-seven were finally used, and 1697 fertile F_1 matings were made, the females being mated to their 'scar' brothers. This represented probably about 70% of the total number available.

When the F_2 emerged the vials were inspected, usually under the microscope, and any which showed one or other of the expected types of males to be missing were set aside for further examination: lethals of course were not certified until all the offspring had emerged in the suspected cultures.

The results are given in detail in Table 1. Of the twenty-seven males seven transmitted mutated sperm. Of these, one gave five; one, three; one, two; and four, one each. The groups of five and three, being all total lethals, may or may not have had their origin in a single spermatocyte, but the group of two were certainly of different origin, one being a total lethal and the other a Minute type of semi-lethal, the flies being small, late-hatching and few. The percentage of mutated sperm is therefore represented as being between a minimum of 0·471 and a maximum of 0·823. (Location tests were not possible on this chromosome.)

Distribution of lethals in time. Two interesting facts emerged from this experiment: (1) the occurrence of two or more mutations in three out of the seven lethal-giving males,

Table 1. *Results of Experiment A*

Mating: scar ♀ × sc^8 sc^{s1} *In S* w^a ♂. Slow-breeding method: same male and female throughout.

Brood	Days	Pair 2	3	4	6	7	8	9	10	11	12	13	15	16	17	18	20	21	23	24	25	27	28	31	32	33	34	36	Total F_1 ♀♀	Lethals	No. ♂♂ giving lethals	% lethals
1	1–7	2	•21	12	15	10	27	11	16	8	17	.	29	20	17	14	•′•″14	5	•22	.	42	25	.	•13	30	5	29	30	434	5	4	1·152
2	7–13	12	.	.	.	31	27	26	.	32	37	41	30	42	28	.	22	19	12	.	49	22	.	••37	32	7	30	48	584	2	1	– (min.)
3	13–20	22	.	.	.	30	•35	2	.	12	63	49	•31	28	15	.	29	.	.	28	35	.	19	32	21	.	38	5	494	2	2	0·405
4, 5	20–30	•29	.	.	.	12	••49	.	.	.	24	.	.	18	5	.	16	.	.	7	8	.	10	.	.	.	7	.	185	5	2	0·540 (min.)
Total F_1 ♀♀		65	21	12	15	83	138	39	16	52	141	90	90	108	65	14	81	24	34	35	134	47	29	82	83	12	104	83	1697	.	.	.
Total lethals		1	1	.	.	.	5	.	.	.	.	.	1	.	.	.	2	.	1	.	.	.	.	3	.	.	.	.	.	14	7	0·823 (max.)
Min. no. of different lethals		1	1	.	.	.	1	.	.	.	.	.	1	.	.	.	2	.	1	.	.	.	.	1	.	.	.	.	.	8	7	0·471 (min.)

• indicates lethals in sperm; ′ and ″ indicates two different lethals (see text).
Minimum percentages are based on number of lethals, known to be different, maximum percentage on total number of lethals obtained.

Table 2. *Results of Experiment B*

Intensive breeding method. Oregon-K males were placed in separate vials with 3–6 new females (sc^8 sc^{s1} *In S* w^a *ct*) every 24 hr.

Days	♂ no. 1	2	3	4	5*a*	5*b*	6	7	8	9	11*a*	11*b*	12	14	15	16	17	18	19	Total F_1 ♀♀ mated	Total F_1 ♀♀ fertile	No. of lethals	% lethals
1	Few or none	5	36	•84	24	14	Few or none	Few or none	62	Few or none	•26	44	61	•67	Few or none	36	56	75	22	612	582	3	0·515
2	Do.	65	109	138	Lost	?	92	95	43	60	Lost	?	91	101	50	79	69	21	30	1043	959	.	.
3	96	?	57	134	.	...	Lost	110	?	?	.	?	52	29	?	138	32	51	?	699	637	.	.
4	+ +	+ +	+ +	+ +	.	+ +	.	?	+ +	+	.	?	17 +	17 +	?	?	?	?	?	34	33	.	.
5	+ +	=	+ +	+ +	.	D	.	+ +	+ +	49	.	56	31	–	(No. ♀)	+ +	+ +	–	+ +	136	136	.	.
6	65	17	59	60	.	.	.	26	=	35 +	.	–	=	–	72	55	52	90	29	560	534	.	.
7	Lost	–	24 +	12	.	.	.	=	49	33	.	–	109	–	51	42	=	58	48	426	405	.	.
8	.	–	21	59	.	.	.	91	52	33	.	–	36 +	–	22	?	85	75	73	547	522	.	.
9	.	...	...	23	.	.	.	19	=	Lost	.	–	24	–	...	...	53	35	14	168	161	.	.
10	.	–	24	14	.	.	.	52	82	.	.	–	47	–	–	24	33	...	?	276	271	.	.
11	.	–	18	16	.	.	.	11	44	.	.	–	21 +	–	–	=	12 +	D	–	122	122	.	.
12	.	–	–	17	.	.	.	–	–	.	.	–	53	–	–	77	–	.	–	147	136	.	.
13	.	–	–	–	.	.	.	–	D	.	.	–	–	D	–	–	–	.	–	.	.	.	.
14	.	–	–	+	.	.	.	–	.	.	.	–	–	.	–	–	–	.	–	.	.	.	.
15	.	–	–	...	.	.	.	...	.	.	.	–	–	.	–	...	–	.	–	.	.	.	.
16	.	–	–	...	.	.	.	–	.	.	.	–	–	.	–	–	–	.	D	.	.	.	.
17	.	–	–	...	.	.	.	–	.	.	.	–	–	.	–	–	–	.	.	.	.	.	.
18–33	.	D18	D31	D24	.	.	.	D?	.	.	.	D28	D32 ±	.	D33 ±	D33 ±	D33 ±	.	.	.	.	.	.
F_1 ♀♀ mated	161	87	348	557	24	14	92	404	332	210	26	100	542	214	195	451	392	405	216	4770	4498	3	0·066

• indicates lethals; ? indicates no record; – indicates no offspring; = indicates gap in sperm production; ... indicates few offspring; D shows day of death.
+ indicates offspring; + + many offspring, not counted; ± indicates the approximate date of death.

and (2) the apparently uneven distribution of the lethals in time. The germ cells used during the first week of life seemed to be the most susceptible of mutation, and those used during the second week appeared to be the most resistant. It seemed possible there might be a rise after the third week. The significance of the results, however, especially as seen in the later broods, was very unclear owing to the short-comings of the method used. The parent flies had been left too long in the same vials, with the result that the number of offspring obtained was much below the possible maximum, while the offspring included in the different broods must overlap considerably, since the females would cease laying when food conditions became unfavourable. Nevertheless, even when these objections were fully weighed the differences in percentages of lethals in successive broods seemed probably significant. Accordingly the next experiment was undertaken with a view mainly to clearing up this point.

Experiment B

This experiment was designed (1) to make it possible to distinguish between the offspring arising from sperm used on each successive day of the entire life of the males; (2) to obtain as large a sample as possible of each male's sperm in order to obtain as large clumps of mutations as possible if any occurred; and (3) to make possible the location of all lethals occurring in clumps (i.e. from the same male). The method used was as follows:

Males of the wild type 'Oregon-K' stock, not more than 24 hr. old, were selected as virgins and placed in separate vials with three or four virgin females 3 or 4 days old. The females used were of a scute apricot stock similar in constitution to that used in the previous experiment but containing the additional marker, cut. After 24 hr. the males were transferred unetherized to fresh vials and a fresh lot of females was placed with each one. The females of the first vials were allowed to lay eggs for 48 hr. altogether in the mating vials, after which they were transferred to fresh vials and allowed to deposit eggs for a further 2 days. This procedure was maintained for at least 25 days in order to ensure that the largest possible sample of sperm would be obtained from each male. Hence the number of 'broods' of each male would be equal to the number of his breeding days, and there could be no overlapping of broods since the mothers of each brood would be different sets of females.

It was intended to mate the whole of the F_1 generation, but unfortunately it was found impossible to cope with all the material, and the offspring of the 4th and 5th days were almost all discarded. There was also a certain amount of loss through overcrowding in some of the parental vials. However, a total of 4770 F_1 females was obtained. These were mated to their scute apricot cut brothers or to males from the scute apricot cut stock. 272 of the cultures produced nothing, leaving a residue of 4498 fertile cultures.

The results of the experiment, which were totally unexpected, are shown in full in Table 2. Only three mutations were obtained. They were from three different males giving an overall percentage of 0·066 on the total number of sperm studied; but they all occurred in sperm used during the first 24 hr. period, i.e. among the first 600 sperm. Other interesting points were: (1) the rapidity with which the entire stock of sperm was used up: after the 12th day a few of the vials gave only a very few offspring. Yet seven of the nineteen males survived beyond the 28th day (an average length of life which compared favourably with that of males of the same stock in later experiments); (2) in spite of the short breeding period of these males, their fertility on the average was as great or greater

than that of males which were bred in the ordinary way in other experiments. The offspring of the 4th and 5th days were estimated to be each at least as numerous as those of the succeeding 6th day, hence the totals given in Table 2 probably represent about four-fifths (or less) of the true fertility totals. This being so, it would probably not be true to say that the males 'became sterile' as a result of intensive breeding; the figures suggest rather that it is possible in the space of 12 days, or in about one-third of the life span, for the *Drosophila* male to exhaust the total amount of sperm capable of being produced during his lifetime. The occurrence of all three lethals in the sperm used during the first 24 hr. period gave some support to the idea that the first batch of mature sperm is particularly sensitive to the influences which favour the production of 'spontaneous' mutations. That these mutations really arose in the mature sperm or at least not before the later stages of spermatogenesis was suggested by the fact that they were all single mutations. In the case of two of them (nos. 4 and 14) the sample of sperm obtained was probably sufficient to indicate the presence of large 'clumps' of mutations if such existed.

From this experiment it also appeared that sperm which is used up as rapidly as it becomes available (as must be the case when the males are given continuous and unrestricted opportunities for mating) may escape the action of mutagenic influences altogether, or that these mutagenic influences might themselves be conditioned by the density of sperm in the testis.

A third experiment was therefore planned with the object of testing these hypotheses as well as of providing a measure of real comparison between the mutation rates of Oregon-K males and of the scute apricot males of Exp. A. It seemed probable that the breeding technique was a highly important factor in affecting variability, and that experiments to give comparable results must conform to some standard method of breeding. The much higher mutation rate of Exp. A might be due either to genetic differences in the marked strains used or to the slow breeding method employed.

Experiment C

In this experiment Oregon-K males were mated individually to scute apricot or scute apricot cut females, one female being placed with each male at the usual temperature of 24° C. The flies were transferred to fresh food on the 4th, 9th, 11th, 15th, 18th, 20th, 22nd, 25th, 29th, 32nd, and 35th days giving twelve broods in all, or approximately two a week (instead of only one a week as in Exp. A). On the 18th day the old females were removed, and a group of two, three or four young females was placed with each male. The old females were left alone for a week in order to deposit their remaining eggs. The surviving ones were returned to their respective mates after a week. The males whose first mates had not survived were left with the same group of females placed with them on the 18th day. By this method it was hoped to obtain from the males as large a number of offspring as was given by the males of Exp. B but spread over the whole lifetime of the fly (as was the case in Exp. A), the sperm being only gradually released. The change of females in the third week would enable the males to get rid of stored sperm and might show a fresh burst of mutation following their retention in the testis.

As will be seen from Table 3, seventeen males gave 5487 F_1 daughters (5288 of which were fertile) over a breeding period of 35 days, or about the same number given by the nineteen males of Exp. B in one-third of the time. Of the seventeen males, seven gave lethals; three gave two each, and four, one each. The pairs of lethals of males 6 and 19

Table 3. *Results of Experiment C*

Mating: $sc^8\ sc^{s1}\ In\ S\ w^a$ ♀ × Oregon-K ♂ (17 ♂♂).
Modified slow-breeding method: new females on 18th day.

Brood	Days	♂ no. 1	2	3	4	5	6	12	14	15	17	18	19	20	22	23	24	27	Total F_1 ♀♀ mated	Total fertile F_1 cultures	Lethals in sperm	% lethals
1	1–4	21	36	43	32	•22	31	54	49	31	40	57	•′ •″ 49	27	28	34	37	40	631	.	3	.
2	4–9	43	45	•39	63	31	•′ •″ 56	15	53	46	35	46	20	33	60	•35	47	52	719	.	4	.
																				1293	(7)	0·541
3	9–11	40	52	27	40	30	41	.	30	61	.	70	58	.	63	38	42	30	622	.	.	.
4	11–15	40	60	.	26	41	30	.	49	45	.	62	41	.	(New ♀) 43	31	57	26	551	.	.	.
5	15–18	(No ♀)*	77	.	28	30	60	.	.	25	.	21	38	.	115	23	35	.	452	.	.	.
																				1565	0	0·000
Change to new ♀♀																						
6	18–20	35	22	.	45	33	41	.	.	115	.	•109	74	.	75	63	70	.	682	.	1	.
7	20–22	•59	98	.	79	73	107	.	.	10	.	42	34	.	.	•80	36	.	618	.	2	.
																				1256	(3)	0·238
8	22–25	76	29	.	61	53	24	.	.	.	.	58	59	.	38	68	29	.	495	.	.	.
9	25–29	48	9	.	47	19	22	.	.	.	.	34	49	.	21	41	38	.	328	.	.	.
10	29–32	29	5	.	54	.	.	.	.	.	.	38	.	.	84	70	.	.	280	.	.	.
11	32–35	59	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	59	.	.	.
12	35–39	50	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	50	.	.	.
																				1174	0	0·000
Total F_1 ♀♀		500	433	109	475	332	412	69	181	333	75	537	422	60	527	483	391	148	5487	5288	.	.
Lethals in sperm		1	.	1	.	1	2	.	.	.	.	1	2	.	.	2	.	.	.	.	10	0·189

• indicates lethals. * This male was left without a ♀ during this period. ′ and ″ indicates that the two lethals from the same ♂ were not identical.

were both composed of one semi-lethal and one total lethal each. The two given by male 23 were separated in time by 13 days and were unlikely to be identical. Thus the percentage on the total number of sperm (5288) was most probably 0·189.

Seven out of the ten lethals occurred among the first 1293 sperm (0·541 %) during the first 9 days of life. This early susceptible period was followed, as in Exp. A, by a resistant period covering the second nine days of life during which no lethals occurred in 1565 sperm. The last three lethals occurred among the 1256 sperm used between the 18th and 22nd days when the old females had been replaced by young ones. The last 1174 sperm gave no lethals.

In the next experiment it was planned to leave the males a longer time with the original females and to change the mating vials more frequently.

Experiment D

The males used in the group D matings were not of the Oregon-K stock, but wild type males extracted from some of the matings of group C in the third generation. A parallel experiment was made with Oregon-K males from stock, but this batch of males was very sterile, short-lived and generally atypical; they gave no mutations in the 1200 females obtained from the fourteen males used. The extracted wild type males on the other hand (though more variable) were fully up to the average standard of fertility and longevity which usually characterize Oregon-K, though they included among their number two unusually fertile and long-lived ones. The complete data are given in Table 4.

In this group the changing of the flies to fresh vials was done three times a week and twenty-five broods were obtained. The males were each paired with a single female. If this female died before the 32nd day, she was replaced by another young female. On the 32nd day, the surviving males were each placed with a group of three young females, which were replaced at short intervals until the death of the males, in order to obtain from them the maximum amount of old sperm. It must be noted, however, that before the 32nd day many of the males had had occasion to be remated owing to the early death of the original females, so that the object of this measure was only partially achieved. A total of 5103 F_1 females from fifteen males were mated, of which 4917 were fertile. Nine of the males gave lethals: one (no. 28) gave three, no. 19 gave two, and seven others gave one each. There were thus twelve lethals in all giving a percentage of 0·244. This is slightly, but not significantly, higher than the figures for the group C matings of 'pure' Oregon-K males.

The distribution of lethals in time showed in general the same characteristics as groups A and C—notably the drop in the incidence of mutations between the 6th and 18th days, reducing the percentage from 0·398 (on 1005 sperm) to 0·052 on 1916 sperm. Between the 18th and 32nd days, which marked the end of life of most of the flies, the percentage rose to 0·319 (on 937 sperm). The last 1059 sperm were obtained from the four most fertile males of the group after they had been placed with three new females, and gave four lethals, three of them occurring during the last quarter of the long life of no. 28. This male had 833 daughters and had also given one visible mutation (a female-sterile rudimentary) in brood 14. It is important to note that the three lethals of this male were located and proved to be widely separated on the chromosome.

Table 5 summarizes the distribution of lethals during successive periods of the lifetime of the flies. In all the slow-breeding experiments the general trend of the periodic

Table 4. *Results of Experiment D*

Modified slow-breeding method: change from 1 ♀ to 2 or 3 from 32nd day.
Mating: $sc^8\ sc^{s1}\ In\ S\ w^a\ ct$ ♀ × extracted wild type ♂ (16♂♂).

Brood	Days	♂ no. 15	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Total mated	Total fertile	Lethals	%
1	1– 4	37	34	39	.	15	6	•51	27	26	31	31	35	37	25	25	30	449	.	.	.
2	4– 5	18	14	23	15	.	.	26	12	12	21	•23	22	27	.	14	28	255	.	.	.
3	5– 6	18	•19	37	35	.	.	45	•21	17	29	18	30	18	.	13	23	323	.	.	.
																			1005	4	0·398
4	6– 8	20	.	53	46	*.	*11	64	36	9	32	18	22	24	*3	40	49	427	.	.	.
5	8–11	41	.	48	49	*13	33	8	45	3	32	26	40	16	37	30	38	459	.	.	.
6	11–13	19	.	30	•33	8	17	.	14	.	18	9	7	15	22	14	21	227	.	.	.
7	13–15	30	.	46	30	9	2	*40	40	*23	33	9	24	23	31	41	36	417	.	.	.
8	15–18	30	.	56	28	5	9	21	28	28	33	18	31	22	25	56	40	430	.	.	.
																			1916	1	0·052
9	18–20	*3	.	27	7	5	.	6	3	4	22	5	.	.	•14	18	27	141	.	.	.
10	20–22	•38	.	41	.	.	.	4	7	23	29	20	.	*.	11	22	24	219	.	.	.
11	22–25	1	.	2	.	.	.	5	43	51	*18	25	1	71	3	12	9	241	.	.	.
12	25–27	.	.	*.	.	.	.	3	24	.	.	4	.	38	.	.	.	69	.	.	.
13	27–29	.	.	21	13	.	.	8	26	.	37	4	.	25	.	.	18	152	.	.	.
14	29–32	.	.	.	36	.	.	12	23	.	35	6	.	19	11	.	•28	170	.	.	.
																			937	3	0·319
15	32–34	.	.	13	17	.	.	.	Lost	.	.	.	.	6	.	.	24	60	.	.	.
16	34–36	.	.	46	62	.	.	.	.	.	.	.	.	44	.	.	86	238	.	.	.
17	36–39	.	.	43	•47	.	.	.	.	.	.	.	.	65	.	.	60	215	.	.	.
18	39–41	.	.	.	30	.	.	.	.	.	.	.	.	32	.	.	13	75	.	.	.
19	41–44	.	.	.	66	.	.	.	.	.	.	.	.	86	.	.	44	196	.	.	.
20	44–47	.	.	.		.	.	.	.	.	.	.	.	•87	.	.	75	162	.	.	.
21–25	47–60	.	.	.	.	.	.	.	.	.	.	.	.	•• 178	.	.	.	178	.	.	.
																			1059	4	0·377
Total:		255	67	525	514	55	78	293	349	196	370	216	212	833	182	285	673	5103	4917	.	.
Lethals:		1	1	.	2	.	.	1	1	.	.	1	.	3	1	.	1	.	.	12	0·244

• indicates lethals in sperm; * indicates death of female and replacement by new one.
From the 15th brood on, males were placed with several new females at short intervals.

differences in mutation rate seems clear enough: the sperm used during the first 6–8 days of the life of the male would appear to be the most susceptible to mutational influences; this is followed by a period of from 8 to 12 days during which resistance to mutation is at its highest (or mutational influences least likely to be present); in later periods the mutation rate again rises, though perhaps not to the level of the first period, though in the longest lived males (D) the output of lethals continued high to the end.

A comparison of the overall mutation rates between marked and unmarked flies shows a considerably higher figure for the *sc* w^a males (A) than for the wild-type males of the other experiments. This difference is in the direction expected, but of course many more experiments are needed before it can be shown to be of a fundamental nature.

Table 5. *Distribution of lethals in males' lifetime*

Exp.	Period of males' life in days	No. of broods	No. of *X*-chromosomes tested	No. of lethals	No. of ♂♂ giving lethals	% lethals (minimum)
A	1– 7	1	434	5	4	1·152
	7–13	1	584	2	1	0·0
	13–20	1	494	2	2	0·405
	20–30	2	185	5	2	0·540
C	1– 9	2	1293	7	5	0·540
	9–18	3	1316	0	—	0·000
	18–22	2	1505	3	3	0·199
	22–39	5	1174	0	—	0·000
D	1– 6	3	1005	4	4	0·398
	6–18	5	1916	1	(1)*	0·052
	18–32	6	937	3	3	0·319
	32–60	10	1059	4	2	0·377
B	1– 2	1	582	3		0·515
	2–12	11	3916	—	—	—
	12–33		No further offspring obtained			

In Exps. A, C and D (slow-breeding methods) the first period always shows the highest mutation frequency and the second period the lowest. The third period is always, and the fourth period usually, higher than the second. In Exp. B (intensive breeding) mutations occurred only among the 1st day's sperm, and the output of offspring practically ceased on the 12th day.

* This male is the same as one of those of the 32–60 day period.

DISCUSSION

(*a*) *The periodicity of mutation frequencies*

Muller (1946) recently reported that the mutation frequency is three to four times higher in the first crop of spermatozoa, accumulated during the male's pre-imaginal life, than in those of a week later, derived from non-virgin males. The results of the present studies agree with Muller's in this respect. Kaufmann (1945), however, states that throughout the life of the male, the susceptibility of the sperm to mutational influences declines consistently with age, so that 32-day-old sperm are less likely to contain mutations than any of the previous age groups studied. There is some contradiction of Kaufmann's findings in the experiments reported here, which may be only apparent when all the data are considered. In the three 'slow-breeding' experiments (Table 5) in which the males were kept with one and the same female during the whole (A), or during half (C and D) of their life, a constant type of periodicity is discernible: an initial high frequency obtaining among the sperm of the first 6–9 days is succeeded by a conspicuous drop in the percentage of

mutations among the crop of sperm obtained between the 6th and 18th days. This period varies somewhat in each experiment owing to the differences in the times of changing the flies to fresh vials, but they all cover roughly the 2nd week in the flies' life. After this period of low frequency the percentage rises consistently; in both A and D the rise is maintained to the end of life; in C, however, it falls again during the last fortnight. When these results are compared with those of Exp. B, in which the breeding method was intensive, it is seen that in the latter experiment only the sperm used during the first 24 hr. contained any mutations; a fact which supports the idea that 'storing' of the sperm, such as prevails to some extent under any method of slow or restricted breeding, was a decisive factor in raising the frequency of mutations in the other three experiments. When the mature sperm is released as rapidly as it becomes available, it seems that mutations are less likely to occur. Thus it would be necessary to know what the breeding methods used in Kaufmann's experiments were, in order to know whether there is any real inconsistency between his results and those reported here.

The fact, however, that storing of the sperm through restricted breeding tends to raise the mutation rate, rather emphasizes the considerable differences found between the frequencies obtaining during the first week of breeding and those obtaining during the second week. The high initial frequency is too marked to be attributed to pre-imaginal storing alone, as Muller has pointed out (loc. cit.). Moreover, it is interesting to note that the amount of storing involved in restricted breeding during the first week of life does not significantly increase the mutation frequency among the first 1000 sperm (as represented by the first thousand offspring obtained): whether these first 1000 sperm were all used within 2 days or were spread out over 9 days the frequencies obtained (for the three comparable sets of males, namely, the B, C and D wild type groups) varied only between 0·3 and 0·5%. Hence it would appear that the effects of storing, in so far as it occurs in slow breeding, only begin to be observed during the third week—that is, after the period of low frequency. Confirmation of these results would go some way to show that the assumption often made, that 'time' in itself (apart from changes associated with it) is an important factor in the production of mutations, is probably fallacious.

It should be pointed out however that the problem is further complicated by the fact that maturation of the sperm is almost certainly affected by rate of sperm release (as will be shown later in this discussion) and the possibility remains that the causative factor in the increased frequency of mutations in the later breeds of the slow-breeding experiments is not prolonged storing of mature sperm as such, but the lengthening out or deferment of the maturation processes. The question of the stage of maturation at which spontaneous mutations are most likely to occur may also be involved in these considerations. But, while the present experiments cannot be regarded as conclusive, they do suggest that it is more usual for mutations to occur in late rather than in early stages of spermatogenesis. For the majority of the lethals obtained in all the experiments were either obtained from different males or else were obviously different ones (Table 1, ♂ 20; Table 3, ♂♂ 6 and 19) or proved by location to be different (Table 4, ♂ 28). When allowance is fully made for the wastage of sperm involved in the economy of fertilization it would seem extravagant to assume that any considerable proportion of the lethals obtained are in fact the sole representatives of large clumps of mutations derived from early immature germ cells.

(b) *Rate of breeding: rate of sperm development*

Some conclusions regarding spermatogenesis in the adult male are suggested by Exp. B. It is often assumed that this process is continued at a fixed and constant rate throughout the life of the fly. Thus Demerec & Kaufmann (1941) in experiments 'planned to determine the length of time after irradiation that males may be bred before they begin to utilize the sperm which was immature at the time of treatment' allowed the treated males to copulate only on the 6th, 12th, 13th, 18th and 24th days of life, leaving them without females on the intervening days. Their conclusion that sperm which was immature at the time of treatment 'does not become available until some time after twelve days' (actually the 19th day) is difficult to reconcile with the results of Exp. B. Since the males of Demerec & Kaufmann had only mated on 5 days altogether, at arbitrarily spaced intervals, it seems possible that the same result might have been obtained if the matings had taken place on five consecutive days. The data shown in Table 2 suggest that there must be a well-defined limit to the capacity of the testis to elaborate sperm, and that when the 'B' males ceased to give offspring their capacity for sperm production at least in effective quantities had been exhausted. The date of sperm exhaustion was as early as the 4th or 6th day for the less fertile flies (nos. 2 and 14), but for the majority the 11th or 12th day of life. Some of the most fertile ones (nos. 4, 7 and 16) gave a very few offspring on the 15th day, and no. 4 continued to put out a very meagre supply up to the 17th day. According to Pontecorvo (1943) 'the testis of the imago.....is divided into three zones: the spermatogonia are limited to the very tip; then follows a region of first spermatocytes, and finally there is a region of later stages of spermatogenesis usually occupying more than half the testis'. Demerec & Kaufmann showed in their experiments that the mature sperm present in the ejaculum on the 2nd day of mating (the 12th of life) was exhausted after four copulations, but that 14 hr. later a fresh supply was available and was sufficient for three copulations. On the 1st day of mating four copulations had not exhausted the supply. Hence on each successive day of mating, in spite of the breaks in the continuity of sperm release, there were progressively fewer spermatozoa available for immediate use. On the 5th day of mating (the 24th of life) it was calculated that the spermatozoa which were immature at the time of treatment (i.e. the 6th day of life) had become mature. If the performance of these irradiated flies be compared, however, with that of the group B males of this paper, it becomes probable that the age of the irradiated flies had little or nothing to do with the timing of the appearance of the more recently matured sperm. In Table 2 it will be seen that as early as the 5th day, and possibly on the 4th day for some of the males (7, 16, 17), there is a clear gap of 24 hr. in the supply of sperm (marked = in the table) usually followed by an increased output on the next day. In male no. 4, though there is no complete gap, on the 7th day there is a very small number of offspring between two normal quantities. In no. 3 the same sort of sequence occurs 2 days later. In male no. 8 there are two clear gaps, on the 6th and 9th days. These gaps certainly do not show merely that the sperm in the ejaculum had been exhausted. It is much more likely that they represent stages where all the mature sperm in the testis had been used and where the germ cells in the earliest stages (those seen by Pontecorvo at the far tip of the testis) had not yet completed their development. The immediate rise in the number of offspring after the gap suggests widespread synchronization of the final stages of maturation. It seems then that it is possible for all the mature sperm present in the testis to be

used up in 5–7 days, *if the young adult is given unlimited opportunities for mating from the time of emergence.* In other words, whether the males are bred intermittently or continuously the supply of mature spermatozoa would last the same number of full breeding days. If this is in fact true it must follow that spermatogenesis does not proceed at a fixed pace irrespective of the rate of sperm release, but that maturation of the spermatogonia is to a great extent conditioned by the release of the mature spermatozoa. Experiments described by Harris (1929) and Hanson & Heys (1929) give some support to this suggestion. Harris irradiated males and mated them individually to one female each. The females were replaced by new virgin females every 4th day. Although this method is described as allowing the males 'to reproduce from day to day' it is essentially a 'slow-breeding' method, since the males would not be able to copulate freely with one female for four successive days. At the same time the opportunities for mating would be greater and more evenly spaced than those allowed to the males of Demerec & Kaufmann, though considerably less than those of the B males of this paper. As would be expected on the above hypothesis, the sperm immature at the time of treatment came into use several days earlier than was the case with the males of Demerec & Kaufmann; the latter gave evidence of the presence of recently matured sperm on the 19th day after treatment (which was also the 5th day of mating and the 24th of life); Harris's flies showed this evidence in the 12–16 day mating period. His figures make it clear that the decisive day was the 12th, i.e. 7 days earlier than Demerec & Kaufmann's flies. Hanson & Heys used the strict pair-mating method, the males being left with one and the same female for 35 days. In these conditions the males were said to be 'continuously mated', but it is obvious that the breeding method was even 'slower' than that used by Harris—hence, on the hypothesis here suggested, the time of appearance of newly matured sperm would be expected to be later than was the case with Harris's flies; it was, in fact, 2 days later than Harris's flies and 5 days earlier than Demerec & Kaufmann's.

It would seem that the number of days necessary to exhaust the supply of mature sperm present in the young *Drosophila* male is in direct proportion to the opportunities for mating with which the male is supplied, and that the maturation of the immature germ cells keeps pace, within the observed limits, with the release of the mature sperm. It seems likely that as long as the mature spermatozoa remain unused the spermatogonia remain largely in a quiescent condition.

It must also be pointed out that the finding by Harris that the proliferation of the germ cells occurs through a system of 'indefinitely reproducing cells functioning like apical cells' appears doubtful in view of the results given by the group B males. It would seem, on the contrary, that the number of mature germ cells ultimately to come into being is predetermined at some early stage in the development of the germ track. The facts suggest that there might be a stage when the mitotic divisions of the primordial germ cells which, according to Sonnenblick (1941), begin about 4 hours before the hatching of the larva, come to an end. At this point a new stage in development would be initiated when a group of these later primordial cells would take part simultaneously in a more specialized movement towards differentiation, to be followed at intervals by other batches of cells. Some such mechanism would in fact be necessary to explain the situation in the mature testis as described by Pontecorvo (1943). Moreover, the existence of such a turning point in the development of the germ track might account for the greater susceptibility of this first-formed batch of sperm to mutagenic influences. Such a situation would be in keeping

with the finding of Packard (1935) that those stages in embryogeny at which some new type of development is initiated are those which are most sensitive to the effects of radiation.

Finally, a word may be said about the effect of intensive breeding on the overall fertility of the males. The suggestion that the males of Exp. B ceased to give offspring after a comparatively short breeding period because repeated copulations had resulted in loss of function in the ejaculatory apparatus, is a plausible one, and is used by Duncan (1930) to explain the sterile period observed in males (a wild-type stock of much greater longevity and fertility than the Oregon-K studied here) after moderately intensive breeding. The finding by Duncan of sperm in the testes of males examined during the sterile period is not conclusive since the conditions in the testis under which copulation may be effective are not known and are probably dependent to some extent on the bulk of the sperm available. Hence it should not be possible, by moderating the breeding method in any way, to ensure that the last batch of sperm in the testis had been used, but only the last batch which occurred in sufficient quantity to be passed into the ejaculum and to be effectively ejaculated. The observations of Demerec & Kaufmann show conclusively that the testes may be full of mature sperm and yet the ejaculum may remain empty for a period of 14 hr., after exhausting its previous supply, a fact which suggests that the process is not one of continuous supply, but is regulated by some form of rhythmic control.

Variations in fertility as expressed in progeny counts (apart from the limitations imposed by the female) are probably based primarily on the initial number of constituent cells which form the embryonic gonad and which Sonnenblick (loc. cit.) has observed to be variable.

SUMMARY

1. Spontaneous mutation frequencies were studied in a series of four experiments involving about 16,500 sperm obtained from seventy-nine male *Drosophila melanogaster* individually mated from emergence to death.

2. The data show that both the overall mutation frequency and the distribution of lethals in terms of the flies' age are affected by the rate at which the flies are allowed to breed. In intensive breeding, mutations were found only among the sperm of the first 24 hr. In slow breeding a well-defined periodicity was apparent: a first period of high frequency was invariably followed by a period of low frequency which was in turn succeeded by a rise usually maintained till death.

3. The data suggest (1) that the first batch of mature sperm to be used is the most susceptible to mutagenic influences, and (2) that the effect of 'storing' the sperm under conditions of slow-breeding becomes apparent in the third week of life.

4. It is probable that the capacity of the individual male to produce sperm is not reduced by intensive breeding, but that intensive breeding may exhaust the supply in about one-third of the life span.

REFERENCES

DEMEREC, M. & KAUFMANN, B. P. (1941). Time required for *Drosophila* males to exhaust the supply of mature sperm. *Amer. Nat.* **75**, 366–79.

DUNCAN, F. N. (1930). Some observations on the biology of the male *Drosophila melanogaster*. *Amer. Nat.* **64**, 545–51.

HANSON, F. B. & HEYS, FLORENCE (1929). Duration of the effects of X-rays on male germ cells in *Drosophila melanogaster*. *Amer. Nat.* **63**, 511.

HARRIS, B. B. (1929). The effects of ageing of X-rayed males upon mutation frequency in *Drosophila* *J. Hered.* **20**, 299–302.

KAUFMANN, B. P. (1945). *D.I.S.* **19**, 58.

MAMPELL, K. (1945). Analysis of a mutator. *Genetics*, **30**, 496–505.

MULLER, H. J. (1945). Age in relation to the frequency of spontaneous mutations in *Drosophila*. *Year Book of the Amer. Phil. Soc.* pp. 150–3.

PACKARD, C. (1935). The relation between age and radiosensitivity of *Drosophila* eggs. *Radiology*, **25**, 233.

PONTECORVO, G. (1943). Hybrid sterility in artificially produced recombinants between *Drosophila melanogaster* and *D. simulans*. *Proc. Roy. Soc. Edinb.*, Sec. B, Biology, **61**, part IV, no. 28.

SONNENBLICK, B. P. (1941). Germ cell movements and sex differentiation of the gonads in the *Drosophila* embryo. *Proc. Nat. Acad. Sci., Wash.*, **27**, 484–9.

CYTO-GENETICAL INVESTIGATIONS IN SOME COMMON CUCURBITS, WITH SPECIAL REFERENCE TO FRAGMENTATION OF CHROMOSOMES AS A PHYSICAL BASIS OF SPECIATION

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(With Twenty-eight Text-figures)

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Introduction

Many species of the family Cucurbitaceae have unisexual flowers and a number of them are also dioecious. The scanty cytological investigations made until now show, however, that, with few exceptions, the morphological sex differentiation so common in this family cannot be correlated with any chromosomal difference in the gametes. Correns, as early as the beginning of this century, showed by breeding experiments that male plants of *Bryonia dioica* are heterozygous for sex (XY) and the female homozygous (XX). Later researches have shown however that no sex chromosome is present in the pollen mother cells of the male plants. Well-defined sex chromosomes have been reported only in a few species of *Trichosanthes* (Kurita, 1939; Nakajima, 1937) and in *Coccinia indica* (Kumar & Deodikar, 1940). Recently it has been pointed out by Gates (1942) that sex chromosomes in many Bryophytes are probably nucleologenic; that is, they possess either a satellite or a secondary constriction. Information about the relation of sex chromosomes to nucleolus organization in higher plants is, however, completely wanting (cf. Gates, 1939). In view of the fact that Schultz, Casparsson & Aquilonius (1940) have shown that the chemical composition of the nucleolus in *Drosophila* is genetically controlled and may be altered,

it would certainly be very interesting to know whether cytological or chromosomal sex differentiation in plants could be correlated with any kind of nucleolar difference.

The application of improved cytological methods in the study of nucleoli in general and with reference to sat.-chromosomes in particular has thrown considerable light on the evolutionary history of nucleoli in different groups of plants (Gates, 1942; Bhaduri, 1944). No work in this direction has yet been done in any member of this family. The list of chromosome numbers in different members of the Cucurbitaceae shows that there is a distinct aneuploid series, such as $n = 7, 8, 10, 11, 12, 13, 16, 20, 21, 22, 24$, etc. Can such aneuploid numbers be correlated with the maximum number of nucleoli present in each species? To test the validity of the theory of numerical correspondence between the maximum number of nucleoli and satellites in a family with aneuploid numbers, the present investigation was undertaken. Such an investigation should not only throw considerable light on the phylogenetic relationships within the Cucurbitaceae but may help in finding fresh evidence of the cytological basis of speciation.

Materials and methods

The material for the present investigation was obtained from the following species:

(1) *Cucumis sativus* L.
(2) *Cucumis Melo* L.
(3) *Trichosanthes dioica* Roxb. (♀).
(4) *Trichosanthes dioica* Roxb. (♂).
(5) *Luffa aegyptiaca* Mill. = *L. cylindrica* M.
(6) *Luffa acutangula* M.
(7) *Coccinia indica* W. & A. (♂).
(8) *Coccinia indica* W. &. A. (♀).
(9) *Momordica charantia* L.
(10) *Cucurbita maxima* Duch.
(11) *Benincasa cerifera* Savi.

For the study of the morphology of chromosomes, root tips were collected from all the above-mentioned species, and for meiotic materials flower buds were collected from *Coccinia indica*, *Cucumis sativus* and *Luffa aegyptiaca*.

Root tips were obtained from germinating seedlings in all except *Coccinia indica* and *Trichosanthes dioica*. In the cases of *Cucurbita maxima*, *Luffa cylindrica*, *Coccinia indica* and *Trichosanthes dioica*, root tips of adventitious roots from the nodes were collected. *Coccinia indica* root tips were secured by striking the root stalk in moist soil.

In all the species investigated it was found that the peak period of division was very short. The best time for fixation was between 9.30 and 10.30 a.m. in the summer and between 10.30 and 11.30 a.m. in the winter months. In adventitious roots of *Cucurbita maxima* and *Luffa aegyptiaca* on the other hand, the peak time of division was from 12.30 to 2 p.m. in the former case and from 6.30 to 7.30 a.m. in the latter.

For fixation of the somatic chromosomes, Levitsky's fixative with the following composition was used: 1% chromic acid and 10% formalin in equal proportion. This gave the best results except in *Luffa aegyptiaca*, *L. acutangula* and *Coccinia indica*, for which the proportion of the components had to be changed to 2 parts of 1% chromic acid to 3 parts of 10% formalin. For fixation of flower buds, Belling's Navashin was most advantageous. Before fixing, the calyx and corolla were removed and the flower buds were pretreated with Carnoy's (Semmens' modification; chloroform:alcohol:acetic acid, 1:3:1).

For root tips and flower buds, paraffin sections were cut 8–12μ thick in the former case and 16–18μ in the latter. The preparations were stained both with Feulgen light-green

(Bhaduri, 1938; Semmens & Bhaduri, 1939) and Newton's crystal violet-iodine methods. Mordanting the slides with 1% chromic acid (La Cour's modification), after potassium-iodide-iodine treatment improved the quality of the preparation. Certain modifications were also made for staining the pollen mother cells while following Newton's crystal-violet-iodine method. In *Luffa aegyptiaca*, for instance, better results were obtained following La Cour's modified crystal-violet-iodine method, but *Coccinia indica* gave best results when the preparations were mordanted for an hour with Levitsky's solution before staining. For *Cucumis sativus*, premordanting with Stockwell's solution in place of Levitsky's solution was found to be indispensable. In all the materials investigated, Belling's aceto-carmine method gave poor results.

Observations were made using Leitz 1·8 mm. oil immersion objective, n.a. 1·32 with aplanatic condenser 1·4; homogeneous immersion and compensating eyepiece ×10. Drawings were made with a camera lucida using Leitz's eyepiece no. 18 and the oil immersion objective, giving magnification of approximately ×3500.

Observations

The chromosomes in all the species of Cucurbitaceae were found to be very small. The observations presented below reveal the presence of a very high number of satellites and a correspondingly high number of nucleoli in the different members of the family. For example, in the male plants of *Trichosanthes dioica* the number of satellites exceeds the diploid number of chromosomes. Such a high number of satellites has not previously been reported in any plant. Similarly, in *Cucumis sativus* which has $n=7$ chromosomes, and in *Luffa aegyptiaca* which has $n=13$, the maximum number of nucleoli in a dyad nucleus was found to be 6 for the former and 10 for the latter species. In some chromosomes, the satellite stalks were so short that the constriction region between the satellite head and the chromosome arm was almost unresolvable, and a slight twisting or swelling of the chromosome made the identity of the satellite obscure. It was only in very well fixed and critically stained preparations that the maximum number of satellites could be observed. In some chromosomes again, for example, in *L. aegyptiaca*, some of the satellites were represented by minute threads alone. Similar observations have been recorded previously by a number of investigators in widely different plant groups (Resende in *Aloe*, 1937; Mensinkai in *Trillium*, 1939; Bhaduri in *Tradescantia*, 1942*a*). The small size of the chromosomes, the extreme minuteness of the satellites and very short length of the satellite stalk, made a proper analysis of the karyotype of the chromosome complement in most of the species of Cucurbitaceae extremely difficult. This explains why morphological details of chromosomes have been missed by previous investigators in members of this family.

Feulgen light-green preparations were chiefly employed for counting the maximum number of nucleoli and maximum number of chromosomes attached to the fused nucleolus either in the prophase nucleus of a root-tip cell or during diakinesis in the pollen mother cell. Due to the high number of nucleoli and relatively small volume of the somatic nuclei, their maximum number could never be determined from the somatic tissue. Owing to the comparatively large volume of the dyad nucleus on the other hand, the maximum number of nucleoli, which is half the somatic number, could be clearly made out from critically differentiated preparations. It must be mentioned here that in all the species, both in root-tip cells and in diakinetic nuclei, the number of chromosomes attached to the fused

nucleolus was always less than the maximum number of nucleoli observed in the dyad nuclei.

Cucumis sativus, $2n = 14$

Fourteen chromosomes were observed in the root-tip cells of this species, as previously reported by Kozhukhow (1925) and Afify (1944).

Fourteen was the lowest diploid number found in any member of the family so far examined. Due to their small number, comparatively longer size, and well-marked constrictions, a proper analysis of the morphology of the chromosomes could be worked out. In four of the seven pairs of chromosomes the secondary constrictions were unusually pronounced. The primary constrictions were hardly distinguishable. That the exaggerated constrictions were nucleolar constrictions could be ascertained from the number of nucleoli in the dyad nuclei of the pollen mother cell, which correspond exactly to the number of such constrictions present in the somatic chromosomes. Further discussion as to the nature of these constrictions, in relation to nucleoli, is presented later in this paper. It is to be noted here that Afify (1944) has missed all these details regarding the morphology of the chromosomes of *C. sativus* and only observes: 'All the chromosomes have subterminal centric constriction excepting one pair which has submedian centric constriction.' The karyotype for this species is shown in Figs. 1, 1*a*, 1*b*, and 2. The seven pairs of chromosomes are classified as follows:

Pair A. The longest pair of chromosomes has two very prominent constrictions, one being much bigger than the other. Both constrictions are nucleolar in nature (Fig. 2).

Pairs B and C. An unusually pronounced constriction exists in each of these pairs, which is taken to be a secondary constriction. The two arms are equal in length. The primary constriction is obscured by the secondary constriction and must be very near it (Fig. 2).

Pair D. Same as chromosomes *B* and *C*, but in this chromosome the two arms separated by the secondary constriction are unequal in length (Fig. 2).

Pair E. Shorter than the previous pairs. The smaller segment of the chromosome is so small that it appears to be a satellite (Fig. 2).

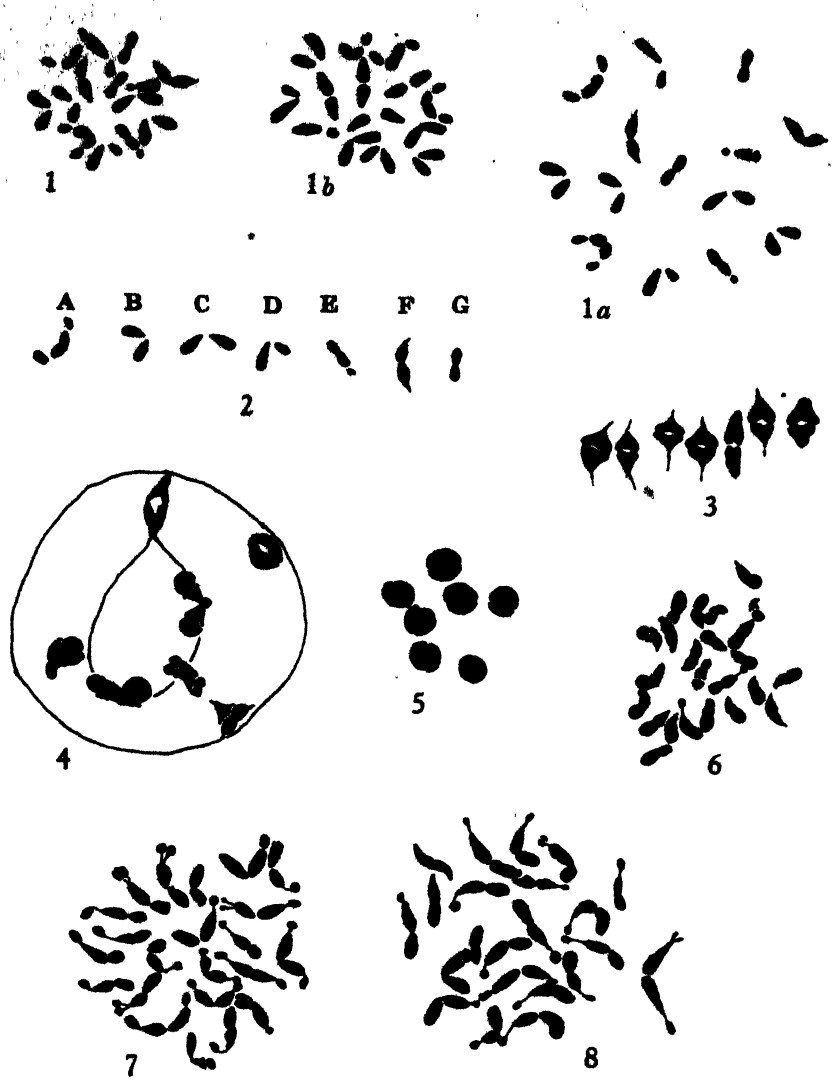
Pair F. Each chromosome has a median primary constriction without showing any indication of the secondary constrictions which are so conspicuous in the chromosomes *A*, *B*, *C*, *D* and *E*.

Pair G. Same as pair *F*, but of smaller size. This is the smallest pair in the complement (Fig. 2).

Seven bivalents were observed as a rule during the first division of the pollen mother cell, corroborating the observations of Heimlich (1927) and Kozhukhow (1925). The pairing and disjunction of the seven bivalents were normal (Fig. 3). Passmore (1930) found two trivalents during meiosis in this species. The figures which he has given in support of this finding, however, indicate bad fixation of the material. Moreover, he has not shown all the chromosomes present in the same figure. It is feared that unsatisfactory fixation led him to misinterpret such figures.

The maximum number of nucleoli was found to be six, and they were of different sizes, one big, one small and two pairs of intermediate and dissimilar size (Fig. 21). The maximum number of bivalents attached to the fused nucleolus during diakinesis was four instead of five (Fig. 4).

Distinct secondary association between bivalents was observed during first division of the pollen mother cell. The maximum association was found to be two groups of three and one free (Fig. 5). As will be seen later, groups of three bivalents are a constant feature



Figs. 1–8.

Figs. 1–5. *Cucumis sativus*. Figs. 1, 1*a* and 1*b*. Somatic metaphase plates showing the 14 chromosomes. Note the marked secondary constrictions in some of the chromosomes. Chromosomes are spaced apart in Fig. 1*a*. Fig. 2. The seven distinct types of chromosomes representing the idiogram of *C. sativus*. Fig. 3. Seven homomorphic pairs and regular disjunction of the bivalents during first anaphase. Fig. 4. Four bivalents attached to the fused nucleolus during diakinesis. Fig. 5. Showing secondary association between bivalents, 2(3)+1. Fig. 6. *C. Melo*, somatic metaphase plate showing 24 chromosomes. Note only two chromosomes with a distinct satellite. Figs. 7, 8. Somatic metaphase plates of *Trichosanthes dioica*, $2n=22$, ♂ and ♀ respectively.

in the pollen mother cell of all the species examined. If we assume the theory of secondary association to be correct, then we find that *C. sativus* is a secondary polypoid species with three as the basic number instead of seven.

Cucumis Melo, $2n = 24$

The number of diploid chromosomes found in this species agrees with the previous observation made by Kozhukhow (1925) and Afify (1944). The somatic chromosomes were found to be much smaller than in *C. sativus* and a detailed analysis of the karyotype could not be made. Only one pair of sat.-chromosomes could be distinguished (Fig. 6). Ervin (1941) also found only one pair of sat.-chromosomes in the somatic complement of this species. Here again Afify (1944) has failed to identify the pair of sat.-chromosomes and mentions only that all the chromosomes have almost median centric constriction. The higher number of chromosomes in this species indicates its polyploid nature. Chromosomes with conspicuous secondary constrictions, as found in *C. sativus*, were not present in this species, and the chromosome types *A*, *B*, *C* and *D* of *C. sativus* were altogether absent in *C. Melo*. The higher number of chromosomes cannot therefore be explained on the basis of auto- or allo-polyploidy. If we assume however that *C. Melo* is derived from *C. sativus* or from a related species by the fragmentation of chromosomes *A*, *B*, *C* and *D* at the locus of secondary constriction, then we can account for the exact number of chromosomes of *C. Melo*. But at the same time we cannot account for the small fragment which will evidently arise by such fragmentation at the secondary constriction region of chromosome *A* of *C. sativus*. Small fragments which should have been evident in the chromosome complement of *C. Melo* were not obtained in reality. It may be concluded that *C. Melo* has been derived from *C. sativus* or a species related to it, first by fragmentation of some of the chromosomes, the fragments acquiring new centromeres spontaneously, followed by subsequent structural rearrangement of the chromosomes, possibly translocations, as visualized by Navaschin (1932).

It is interesting to note that some of the chromosomes of *C. Melo* showed the presence of thread-like projections at their pointed ends. Such an appearance one would expect if one assumes that these chromosomes are derived by fragmentation of some of the chromosomes of *C. sativus* at the secondary constriction region, the fine thread-like structure representing a portion of the secondary constriction region of a chromosome. The pair of sat.-chromosomes in *C. Melo* can be correlated to the pair *E* of *C. sativus*. Additional evidence to the above conclusion has been obtained from an analysis of the maximum number of nucleoli and their size differences in *C. Melo* as compared with *C. sativus*. In *C. Melo* the maximum number of nucleoli in a dyad nucleus was found to be 10 (Fig. 22), a number much higher than the theory of numerical correspondence between satellites and nucleoli demands, because there is only one pair of satellited chromosomes observed in the somatic complement of *C. Melo*. If, on the other hand, we assume fragmentation of chromosomes *A*, *B*, *C* and *D* at the secondary constriction region, then the fragments, A_1A_2, B_1B_2, C_1C_2 and D_1D_2, being portions of nucleolar chromosomes and each having a portion of a secondary constriction, will be capable of organizing separate nucleoli (McClintock, 1934; Bhaduri, 1943). The size of these nucleoli will be smaller than that organized by the entire chromosomes *A*, *B*, *C* and *D* of *C. sativus* (cf. Figs. 21, 22). It may be argued that as the total amount of chromatin substance in a nucleus is constant in a species the total amount of nucleolar substance should also be constant. Actual measurements show that the total volume of nucleolar substance in *C. Melo* and *C. sativus* is almost the same, although the maximum number of nucleoli in the two species is 6 and 10 respectively.

It is to be pointed out here that fragmentation of chromosome A would produce three segments instead of two, namely, A_1, A_2 and A_3. While A_1 and A_3 will organize a single nucleolus each, the middle segment A_2 will be capable of organizing two nucleoli, one on either end. In the latter event the maximum number of nucleoli in *C. Melo* should be twelve in a dyad nucleus. Whether all these nucleoli could be observed in a nucleus is not only a matter of chance but will also depend on the exact locus of breaks. The presence of 10 nucleoli in *C. Melo*, which cannot be explained either on the basis of polyploidy or non-homologous interchange between nucleolar and non-nucleolar chromosomes, strongly supports the above conclusion of fragmentation of particular chromosomes at nucleolar loci.

Trichosanthes dioica, $2n=22$

Male. The somatic number of chromosomes observed from the root-tip cells corroborates the previous observations made by Banerjee & Das (1937). The chromosomes in this species were very small, and the primary constrictions in almost all were median or submedian (Fig. 7). The size differences between chromosomes were not very well marked, although a few were found to be definitely smaller. In properly fixed and critically stained preparations almost all the chromosomes revealed the presence of a satellite—a very interesting feature. The satellited chromosomes had very prominent knobs and threads, and all were satellited except one pair. Further, three pairs of chromosomes showed the presence of a satellite at each end. Thus the total number of satellites becomes even higher than the number of chromosomes. The maximum number of satellites found in this species amounted to 26 (Fig. 7).

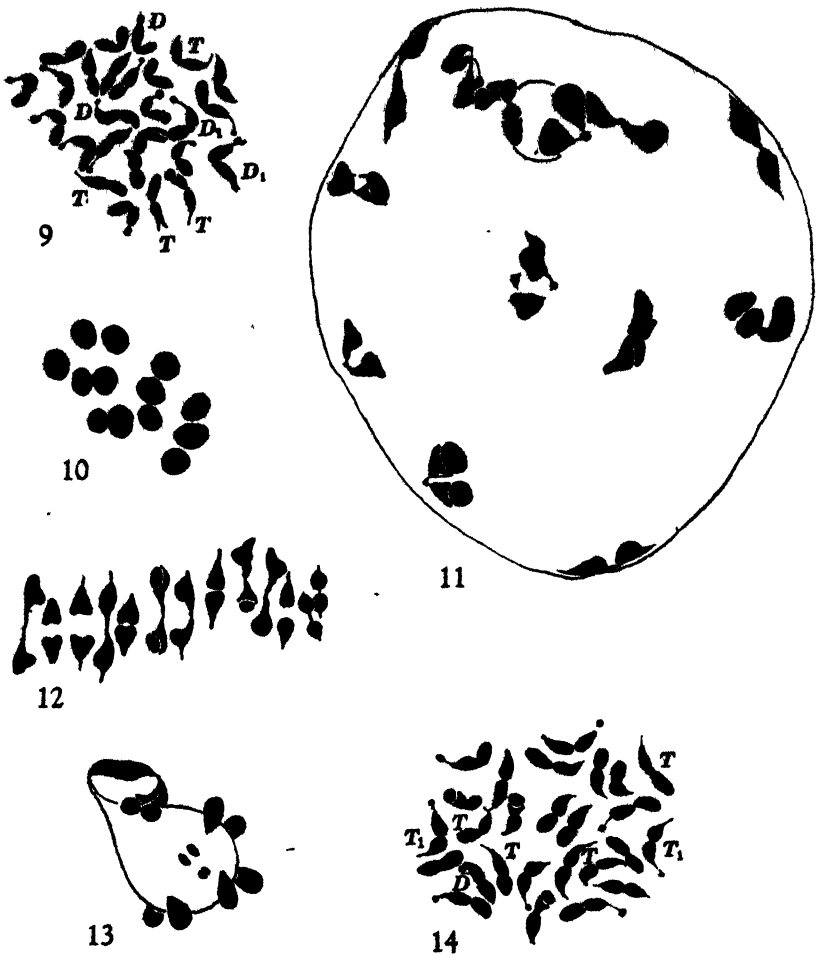
As material for meiotic stages was not available, it could not be ascertained whether these sat.-chromosomes are nucleolar or not. Due to early fusion of nucleoli and small size of the chromosomes, the counting of the maximum number of nucleoli or the number of chromosomes attached to the nucleolus during prophase in the root-tip cells did not throw much light on this point.

Female. The diploid number of chromosomes was the same as in the male plant. Here also the number of satellites was very high. Eighteen chromosomes were satellited. Two of these again possess satellites at either end. These two chromosomes were found to be slightly bigger than the rest. Thus the total number of satellites comes to 20 (Fig. 8). The difference in karyotype between the male and the female plant lies therefore in the total number of satellites present in each complement. Any other differences, if present, were not detectable.

Luffa aegyptiaca, $2n=26$

Twenty-six chromosomes were found in the root-tip cells, as also previously observed by Whitaker (1930, 1933) and Morinaga, Fukushima, Kano, Maruyama & Yamasaki (1929) (cf. Table 1). Of the 26 chromosomes, 12 showed the presence of distinct satellites (Fig. 9). As in other members of this family, the chromosomes were very short, without showing any marked size differences. Almost all the chromosomes had a median or submedian primary constriction. The satellite knobs were extremely small. Besides the presence of 12 distinct sat.-chromosomes, 4 other chromosomes (T) gave indications of their satellited nature. Further, a few other chromosomes showed the presence of a fine thread-like structure alone at the end of a chromosome (Fig. 9). It is extremely difficult at present to state definitely whether these thread-like structures are minute satellites or not.

In the microsporocyte, thirteen bivalents were clearly observed during the heterotypic division, the number corresponding to that reported by previous authors (cf. Table 1). Passmore (1930), however, found eleven bivalent chromosomes in the pollen mother cell of this species. After observing quite a large number of first-division metaphase plates



Figs. 9–14.

Figs. 9–14. *Luffa aegyptiaca*. Somatic metaphase plate showing 26 chromosomes with 12 distinct satellites, some doubtful ones marked '*D*', others which have only the satellite threads without any knobs, marked '*T*'. Two chromosomes D_1, have distinct satellites at one end and a doubtful satellite on the other. Fig. 10. First division metaphase plate showing thirteen bivalents in secondary association of 3(3) + 2(2). Fig. 11. Diakinesis with thirteen bivalents. Fig. 12. First division anaphase, thirteen bivalents disjoining normally. The chromosomes are drawn slightly apart from one another. Fig. 13. Diakinesis; note four bivalents attached to the nucleolus. Fig. 14. Somatic metaphase plate of *Luffa acutangula* showing 26 chromosomes. Note eight distinct satellites, one doubtful satellite marked '*D*' and some distinct chromosomes having the satellite threads only at one end, marked '*T*'. Two chromosomes, T_1, have satellites at one end and at the other end have the satellite thread only.

a distinct secondary association among the bivalents was confirmed. The maximum secondary association was found to be three groups of three and two groups of two (Fig. 10). This indicates that *L. aegyptiaca* is a secondary polyploid, the basic number being 5 instead of 13. From diakinesis (Fig. 11) and anaphase (Fig. 12) it was observed that a heteromorphic pair of chromosomes was not present in this species. Normal pairing and regular disjunction of the 13 bivalents were evident. During first anaphase, 13 bivalents could

be seen separating, half of them passing to each pole. Two bivalents were smaller than the rest (Fig. 12).

From Feulgen light-green preparations it was found that each dyad nucleus contained 10 nucleoli (Fig. 23), which means that the maximum number of nucleoli in each somatic nucleus should be 20. Such a high number, however, could not be corroborated from somatic nuclei. The number of chromosomes attached to the fused nucleolus in a somatic nucleus was also found to be less than 20. In a diakinetic nucleus only 4 bivalents could be distinguished attached to the fused nucleolus (Fig. 13).

This apparent anomaly of the theory of numerical correlation between the maximum number of nucleoli in a species on the one hand and the total number of sat.-chromosomes present in the somatic complement on the other, could be explained, if we assume that all those chromosomes which were considered as doubtful cases of sat.-chromosomes were true nucleolar chromosomes. Occurrence of such minute thread-like satellites has been previously reported in a number of widely different plant genera (Mensinkai (1939) in *Trillium*; Resende (1937) in *Aloe*; Sato (1937) in *Howarthia*; Bhaduri (1942*a*) in *Tradescantia*, etc.).

Luffa acutangula, $2n=26$

This has also 26 chromosomes in root-tip cells. McKay has also observed the same number of chromosomes in this species (cf. Table 1). The chromosome complement appears almost like that of *L. aegyptiaca*. The maximum number of satellites was 8 in this case (Fig. 14), but there were indications of other chromosomes being satellited also, as described for *L. aegyptiaca*.

Coccinia indica, $2n=24$

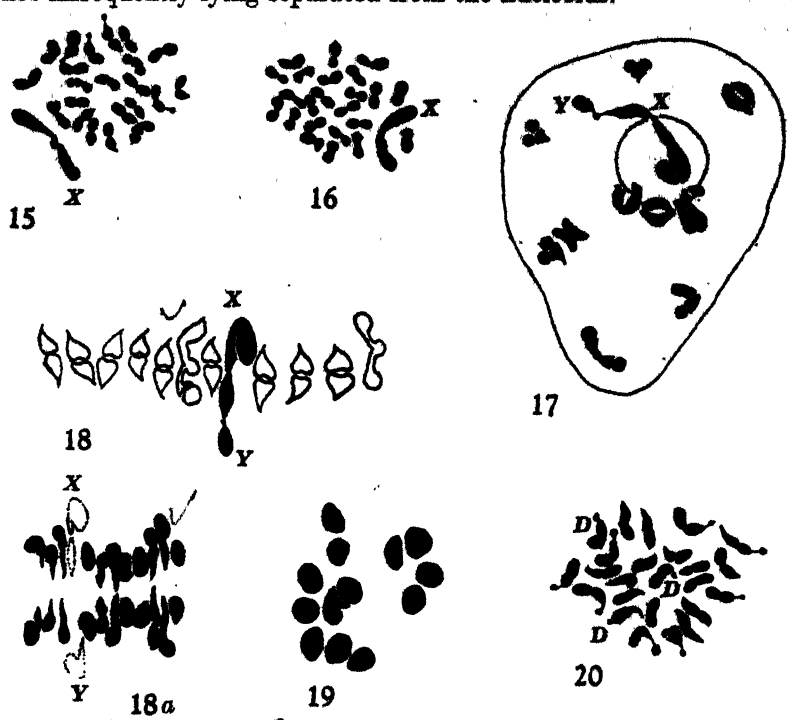
Male. Twenty-four was the diploid number of chromosomes determined for this species, which corroborates the previous observation of Sutaria (1936), but Kumar & Deodikar (1940) have reported 26 chromosomes (cf. Table 1). Of the 24 chromosomes, 4 were satellited (Fig. 15); two others showed an indication of satellites, but a definite statement about the reality of their satellitic nature cannot be made at this stage. All the chromosomes in this plant were found to be very small, except one which was much longer than the others (Figs. 15, 16), nearly three to four times the length of other chromosomes, as will be evident from the measurements given below:

X-chromosome	$4{\cdot}5$–5μ
Autosomes	$1{\cdot}3$–2μ

This chromosome always stained more deeply than the rest and was found to be absent in the female plant. The homologue for this long chromosome could not be distinguished clearly in the somatic complement, because all the remaining chromosomes were almost the same size and shape. During meiosis the presence of a distinct heteromorphic pair consisting of the large X-chromosome* and a much smaller Y could be established (Figs. 17, 18 and 18*a*). The X had a primary constriction in the middle and a secondary constriction near the primary one. All the other chromosomes had median primary constrictions (Figs. 15, 16).

* There is a possible conflict of usages as regards the naming of the X- and Y-chromosomes. The Y-chromosome is generally smaller than the X, but on the other hand it has been customary, where there are two similar sex-chromosomes in the female, to call them X, even if the Y is larger. To avoid confusion it therefore seems desirable to call the large sex chromosome in *Coccinia indica* Y and the small one X. Note added by R.R. Gates.

During the first division of the pollen mother cell, 12 bivalents could easily be counted from the metaphase plate (Fig. 19). Examination of meiotic stages showed that they all paired and disjoined regularly (Figs. 18, 18*a*). The presence of a distinct heteromorphic pair of chromosomes was evident throughout the meiotic cycle. The secondary constriction of the *X*-chromosome, which was not so well marked in the root-tip cells, could be clearly distinguished during diakinesis and first division anaphase (Figs. 17, 18, and 18*a*). The heteromorphic pair of chromosomes, *XY*, was often found attached to the fused nucleolus during early stages of meiosis. In root-tip cells, on the other hand, the *X*-chromosome was found not infrequently lying separated from the nucleolus.



Figs. 15–20.

Figs. 15–20. *Coccinia indica* ♂. Figs. 15, 16. Somatic metaphase plates showing 24 chromosomes, of which four have satellites. Note the sex chromosome with a secondary constriction near the middle. Fig. 17. Diakinetic stage showing three homomorphic and one heteromorphic pair of bivalents attached to the nucleolus. Note the nucleolar region of the *X*- and *Y*-chromosome. Figs. 18, 18*a*. First anaphase, showing the separation of *XY*. Note the secondary constriction of *X*. Fig. 19. First division metaphase, showing twelve bivalents. Note the big pair of sex chromosomes in the middle. Fig. 20. Somatic metaphase plate of *C. indica* ♀, showing 24 chromosomes. Note the eight distinct satellites and three doubtful ones marked '*D*'.

The maximum number of nucleoli in a dyad nucleus was found to be 8 (Fig. 24). During diakinesis four bivalent chromosomes including the heteromorphic pair were seen attached to the fused nucleolus (Fig. 17).

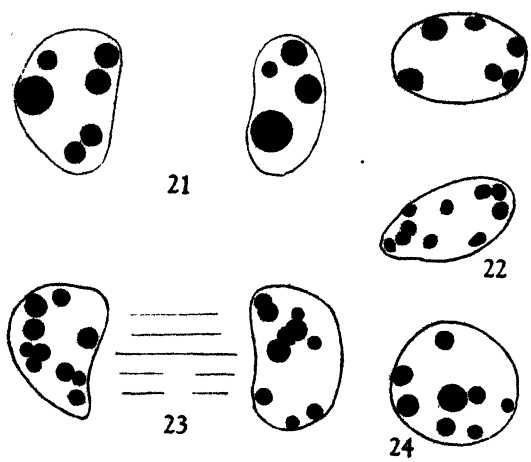
Female. In the female plant 24 chromosomes were present in the root-tip cells. Kumar & Deodikar (1940), on the other hand, have reported 26 chromosomes for this plant (cf. Table 1).

The chromosomes were very small and all were of nearly equal size. Eight were definitely satellited and a few others showed the indication of satellites (Fig. 20). Neither the long *X*-chromosome of the male plant nor the presence of a pair of sex chromosomes could be made out in the somatic complement of the female plant of *C. indica*.

Benincasa cerifera, $2n = 24$

The present determination of the somatic number of chromosomes was found to be 24. Whitaker (1933) observed 12 bivalents in the pollen mother cells of this species (cf. Table 1).

Of all the Cucurbitaceae examined so far, the chromosomes of *Benincasa cerifera* were the longest. Four sat.-chromosomes could be distinguished (Figs. 25, 26). Their knobs were very conspicuous in comparison with sat.-chromosomes found in other species of Cucurbitaceae. The satellite stalks were, however, on the contrary very short, so it became very difficult to distinguish all the sat.-chromosomes in a metaphase plate. Nine other chromosomes showed indications of their satellited nature (Fig. 26), because in addition to the primary constriction other constricted regions were observed in each of these chromosomes which in all probability represented a true secondary constriction.



Figs. 21–24. Showing the maximum number of nucleoli in the dyad nuclei of *Cucumis sativus*, *C. Melo*, and *Coccinia indica* (♂) respectively. ×1750.

Momordica charantia, $2n = 22$

Twenty-two chromosomes were observed in the root-tip cells of this species, which corroborates the previous observation of McKay (cf. Table 1). Because of the small size of the chromosomes, a proper analysis of the karyotype of this species could not be made at present. Two distinct sat.-chromosomes were observed (Fig. 27).

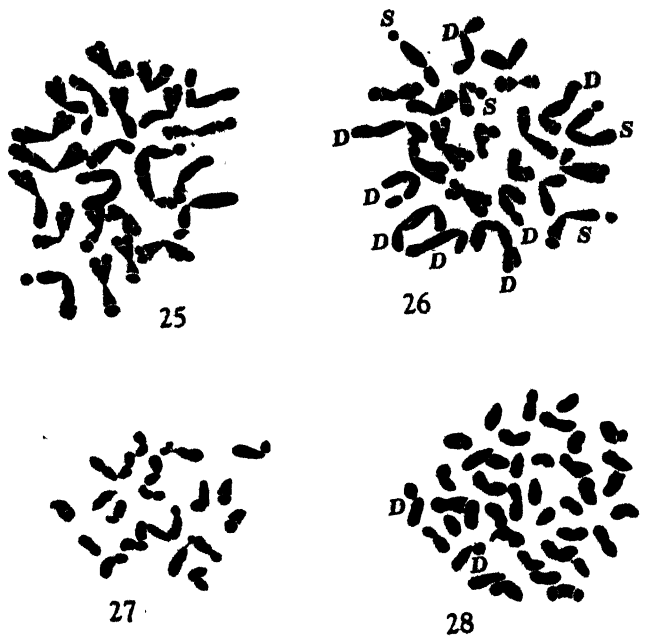
Cucurbita maxima, $2n = 40$

Forty was the diploid number of chromosomes observed in the root-tip cells of this species. The number corroborates that recorded by Castetter (cf. Table 1), but 48 chromosomes in the somatic cells of this species have been reported by Kozhukhow (cf. Table 1). According to Rau (1929), 24 is the diploid number (cf. Table 1). Two of the chromosomes had a very clear satellite, and indications of satellites were observed in other chromosomes (Fig. 28).

Polysomaty

Frequent occurrence of polysomatic cells was observed in *Cucurbita maxima*, *Cucumis Melo* and *Luffa aegyptiaca*. Kozhukhow (1925) saw occasional disomatic cells in root tips of *Cucumis sativus*, *C. Melo* and *Cucurbita Pepo*. He thought that this was induced by

temperature fluctuation at the time of germination. During the present investigation it has been observed that polysomaty is of regular occurrence in this family. Ervin (1941) has also reported its frequent occurrence in this family and mentioned that the influence of temperature in inducing polysomaty is of minor importance, because there is only a small increase of polysomatic cells in the periblem after such treatment. It has been observed during the present investigation that disomatic cells frequently occur intermixed with monosomatic cells in the root-tip tissue, but occasional tetrasomatic cells also appear among them. The polysomatic cells are generally well marked a short distance behind the primordial meristem and appear to be present only in the periblem. Ervin has reported that polysomatic cells occur rarely in the dermatogen and plerome. Polysomatic cells in



Figs. 25–28.

Figs. 25, 26. Somatic metaphase plates of *Benincasa cerifera* showing 24 chromosomes with four distinct satellites. Note the four distinct satellited chromosomes (*S*), and eight doubtful satellites (*D*). Fig. 27. Somatic metaphase plate of *Momordica charantia* showing 22 chromosomes with two satellites. Fig. 28. Somatic metaphase plate of *Cucurbita maxima* showing 40 chromosomes. Note the two distinct satellites marked '*D*'.

Cucurbitaceae are bigger than monosomatic cells and the phenomenon of somatic pairing is of common occurrence in dividing polysomatic cells. The phenomenon of polysomaty has attracted considerable attention of cytologists in recent times (cf. Berger, 1941). A detailed discussion on this subject is postponed for a future publication when more observations on this aspect will be forthcoming.

Discussion

(i) *Numerical correlation between maximum number of nucleoli, satellites and secondary constrictions*

Since the discovery first made by Heitz (1931) and McClintock (1931), a number of investigators have confirmed that satellites and secondary constrictions are the only loci of origin of nucleoli. As the number of satellites or the secondary constrictions in the

chromosome complement of a species is fixed, it follows that the maximum number of nucleoli in a species must also be constant. The validity of this generalization has however been questioned from time to time. The origin of a nucleolus at a particular locus of a particular chromosome, that is, at a satellite stalk or secondary constriction, has been

Table 1

Name of plant	n	$2n$	Reported by
Benincasa cerifera, Savi.	12		Whitaker (1933)
B. hispida		24	McKay (1930)
Bryonia alba	10		Von Bönicke (1911), Meurman (1925)
B. dioica	10		Strasburger (1910), Meurman (1925)
Bryonopsis laciniosa		24	McKay (1930)
Citrullus colocynthis L. Schrad.	11		Whitaker (1933)
C. vulgaris	11	22	Kozhukhow (1925)
Cucurbita Pepo	12	24	Lundegårdh (1914)
C. Pepo L. var. *pomiformis* Alef.	20	40	Kozhukhow (1925)
C. Pepo L. var. *citrullina* Alef.	21	42	Kozhukhow (1925)
C. Pepo var. *Connecticut* Field	20	40	Castetter (1926)
C. Pepo (Jersey White Bush)	20		Passmore (1930)
C. Pepo (English vegetable marrow)	20		Passmore (1930)
C. Pepo var. *Orange gourd*	20		Whitaker (1933)
C. moschata Duch.	24	48	Kozhukhow (1925), Castetter (1926)
C. moschata var. *Calhoun*	20		Whitaker (1933)
C. maxima Duch.	24	48	Kozhukow (1925)
C. maxima	20	40	Castetter (1926)
C. maxima		24	Rau (1929)
C. ficifolia		42	McKay (1930)
C. foetidissima		42	McKay (1930)
C. palmata		42	McKay (1930)
C. melanosperma A.Br.	20		Whitaker (1933)
Cucumis sativus Linn.	7	14	Kozhukhow (1925), Heimlich (1927)
C. Melo L. var. Gr. *melitensis* Alef.	12	24	Kozhukhow (1925)
C. Melo var. Golden Beauty	12		Whitaker (1933)
C. Melo var. Persian	12		Whitaker (1933)
C. anguria	11	22	Whitaker (1930)
C. dipsaceus		24	McKay (1930)
C. metuliferus		24	McKay (1930)
C. myriocarpus	12	24	Whitaker (1933)
Coccinia hirtella		24	McKay (1931)
C. indica		24	Sutaria (1936)
C. indica W. & A.	13	26	Kumar & Deodikar (1940)
Cyclanthera pedata	16	32	Whitaker (1933)
Ecballium elaterium	12	24	Whitaker (1933)
Echinocystis (*Micrampelis*) *lobata*	16		Kirkwood (1907)
Luffa acutangula		26	McKay (1930)
L. aegyptiaca (*cylindrica*)	13	26	Morinaga *et al.* (1929), Whitaker (1933)
L. cylindrica	11		Passmore (1930)
L. gigantea	13	26	McKay (1931)
L. marylandica		26	McKay (1930)
Melothria abyssinica Naud.	12		Whitaker (1933)
M. punctata	12	24	Whitaker (1933)
Momordica balsamina Linn.	11		Whitaker (1933)
M. charantia		22	McKay (1930)
Sicyos angulata		24	McKay (1930)
Trichosanthes anguina	11		Banerjee & Das (1937)
T. cucumeroides	22	44	Sinoto (1929), Nakajima (1937), Sugimoto (1928)
T. japonica	11	22	Sinoto (1929)
T. dioica	11	22	Banerjee & Das (1937)
T. multiloba	11	22	Kurita (1939)

doubted by some investigators. Sato (1938), for instance, found in *Griffinia blumenavia*, a heptaploid species with $2n = 77$ chromosomes, no satellites at all in the somatic complement. The same author (1939) could not find any sat.-chromosome or secondary constriction in *Scilla sibirica* and concluded that the nucleolus is organized around the primary constriction of chromosome 5. The presence of non-nucleolar constrictions in chromosomes, besides the primary ones, has also been recorded from time to time by

a number of investigators. For example, Sato (1936, 1938) has recorded the presence of such non-nucleolar constrictions in species of *Scilla*, *Zephyranthes*, *Cyrtanthus*, *Habranthus* and *Narcissus*; Fernandes (1936) and Sikka (1940*a*) in *Narcissus* and so on. Matsuura (1938), not finding on the one hand any nucleolus-forming region either in *Trillium* sp. or in *Paris* sp., and observing on the other hand the formation of micronuclei with nucleoli in the pollen mother cell, concluded that every chromosome is nucleolar in nature. The usual observation that particular chromosomes are concerned with the formation of nucleoli he has interpreted on the basis of differential amphiplasty.

In recent years, improved technique and careful observation have proved that many of the previous observations which were found contradictory to the theory of numerical correlation between the maximum number of nucleoli and the number of satellites or secondary constrictions present in a chromosome complement, are not correct (Bhaduri, 1942*a*, *b*, 1943; Pathak, 1940*a*, *b*). In *Scilla peruviana* for instance, while Sato (1936) observed only two nucleoli but more than two secondary constrictions in the somatic complement, Bhaduri (1943) has shown that corresponding to 8 secondary constrictions and satellites present in the somatic complement of this species there were also 8 nucleoli in the somatic nuclei. In *Scilla sibirica* also, Sato (1939) found no satellite or secondary constriction in the somatic complement; the pair of nucleoli, he claimed, are organized at the primary constriction of chromosome 5. This observation has later been proved to be inaccurate, and the error has been shown by Bhaduri (1944) to be due to the fact that the secondary constriction is located very near the primary one, which Sato failed to distinguish, due to the limitations of the technique he employed. The same explanation holds good in *Tradescantia* spp., where an exact correlation between the number of nucleoli and the number of satellites and secondary constrictions was established (Bhaduri, 1942*a*). According to Darlington & Upcott (1941), 'In *Tulipa*, as in *Tradescantia* and *Trillium*, there are no regular nucleolar organisers and in consequence no regular nucleolar constrictions. Nucleoli are attached to chromosomes terminally, if at all, and trabants, though sometimes seen in root-tips, are not found at all in pollen-grain mitosis. All constrictions are therefore centric....'

The present investigation on Cucurbitaceae has revealed further interesting features. In most of the species examined a very high number of satellites and correspondingly high number of nucleoli were observed. The maximum number of nucleoli could only be determined, however, in the dyad nuclei. The number in the somatic nuclei was always found to be less than the maximum number of nucleoli determined for the species in question. Whenever the chromosome morphology of the somatic complement of a species could be brought out clearly, a close correspondence between the number of nucleoli and the number of satellites or secondary constrictions present in that species could be established. The presence of a smaller number of nucleoli in the somatic nuclei has to be explained therefore as due to the early fusion of nucleoli in the comparatively smaller volume of the nucleus. In the dyad nucleus, due to larger volume, it was possible to count the maximum number of nucleoli because the chance of fusion between the nucleoli was not so great. It may be suggested, therefore, that before making any definite statement regarding the maximum number of nucleoli present in a species, it is indispensable that both the somatic and meiotic materials be examined.

As mentioned already, in most of the species of Cucurbitaceae examined, the satellites as well as the chromosomes are so small and their numbers so high that an exact deter-

mination of the number of satellites was extremely difficult. Where the number of satellites present in a chromosome complement was found to be less than the number demanded by the theory, it was observed that in most of these cases there were indications of other satellites besides the distinct ones. Further in some species, namely in *Luffa aegyptiaca*, *L. acutangula*, etc., besides the satellites which were indistinct, other chromosomes showed the presence of only a thread-like structure without a knob, at the end of a chromosome. Such satellites are not uncommon in plants and have been recorded before by Bhaduri in *Tradescantia* (1942*a*), Mensinkai in *Trillium* (1939), Resende in *Aloe* (1937), Sato in *Howarthia* (1937), etc. In species like *Benincasa cerifera* it was observed that the satellite stalk is so short that a slight twisting or swelling of the chromosome could easily obscure the identity of some of the satellites.

For all these reasons, it was not possible to make out a full analysis of the karyotype of all the species of Cucurbitaceae studied. In *Cucumis sativus*, where a proper analysis of the karyotype was determined, it was found that a distinct correlation exists. In this case the maximum number of nucleoli, six in the dyad nucleus, corresponds exactly with the number of secondary constrictions and satellites present in the chromosome complement. In *Luffa aegyptiaca*, twelve distinct satellites were observed, four others showed appendages which looked like trabants, and a few others showed satellite threads only, without the knobs. An exact numerical correspondence could thus be established in *Luffa aegyptiaca* also. In *Coccinia indica*, however, there appears some anomaly, because eight nucleoli were observed in the dyad nucleus of the pollen mother cell, whereas only two satellites and a secondary constriction were found in the chromosome complement of the male plant. In the chromosome complement of the female plant as many as 8 distinct satellites were clearly observed. Whether this karyological difference between the male and the female plants is real or apparent cannot be said with certainty at this stage. If we assume however that fragmentation of chromosomes at the secondary constriction region, as shown in *Cucumis Melo*, has produced a higher number of chromosomes in different members of the family Cucurbitaceae, then it appears quite probable that the minute stalks representing portions of secondary constrictions may easily have been obscured due to effects of fixatives and other treatments. Such fragments, although they may not show the presence of fine threads at their ends, may still continue to be nucleologenic. In view of the above explanation the high number of nucleoli in *Coccinia indica* becomes intelligible.

The present observation shows that in most of the cucurbits with such high numbers of satellites and nucleoli there exists the same numerical correspondence between nucleolar number and the number of satellite and/or secondary constrictions as found in various other families.

(ii) *Maximum number of nucleoli and nucleolar constrictions of chromosomes with reference to polyploidy and aneuploidy*

Recent work on widely different plant groups has shown that the number of nucleoli in a species does not necessarily indicate the degree of polyploidy, as was originally considered by De Mol (1928), because many well-known diploid plants are found to have 4 nucleoli instead of 2. The increase in the number of nucleoli in a diploid species had been explained later as due to allo-polyploidy, and some diploid species with four nucleoli were proved to be secondary polyploids (Nandi, 1936; Pathak, 1940*a*, *b*: Iyengar, 1939; Sikka, 1940*b*; Srinath, 1940; Ramanujam, 1938).

It has been realized, however, that numerical polyploidy and secondary polyploidy alone could not explain the presence of a high number of nucleoli in diploid species with low chromosome numbers, as in *Paeonia* (cf. Sinoto, 1938). Recent observations by Bhaduri (1942*a*, *b*, *c*, 1944), employing improved technique, have cleared up the position still further; and it is now definitely established that besides polyploidy, structural changes of chromosomes, such as non-homologous segmental interchange between nucleolar and non-nucleolar chromosomes, may also lead to increase in the number of nucleoli in a species. McClintock (1934) also found evidence of such a phenomenon in X-rayed progenies of *Zea Mays*.

From the present investigation in the Cucurbitaceae it will be seen that although there is direct evidence of secondary polyploidy in some species, presence of such a high number of nucleoli in most of the species cannot be explained on the basis of either numerical or secondary polyploidy or even by segmental interchange between non-homologous chromosomes. Taking the case of *Cucumis sativus*, for example, which is interpreted as a secondary polyploid species with the basic number 3, the 6 nucleoli in the gametes could not be explained on the basis of secondary polyploidy alone. The presence of two unequal secondary constrictions at the end of chromosome '*A*', can be explained either on the basis of previous interchange between nucleolar and non-nucleolar chromosomes, or by assuming that secondary constrictions may differentiate anew and spontaneously in the body of the chromosomes.

In *Trichosanthes dioica* again, in the somatic complement, as many as 26 satellites in male plants and 20 in female plants were observed. From an analysis of the morphology of the somatic chromosomes it could be seen that there might have been non-homologous segmental interchange in *T. dioica*. Although the maximum number of nucleoli in this species has not yet been determined, it will be evident that neither polyploidy nor segmental interchange can explain the presence of such a high number of satellites in this species. In *Luffa aegyptiaca* an exact correspondence between the maximum number of nucleoli and nucleolar chromosomes could be established. Although this species has been described as a secondary polyploid with a basic number 5, the presence of ten nucleoli in the dyad nuclei could not be explained on the basis of secondary polyploidy alone.

A solution of the problem was obtained when we compared the cytological findings in *Cucumis sativus* and *C. Melo*. It has already been mentioned that the increase in the chromosome number from $n=7$ in the former to $n=12$ in the latter, could be explained if we assume fragmentation of chromosomes *A*, *B*, *C* and *D* of *C. sativus* at the loci of extraordinarily exaggerated constrictions, each fragment subsequently acquiring a centromere *de novo* (Koller, 1932; Bhaduri, 1944). An almost proportionate increase in the number of nucleoli in *C. Melo* confirms the above view. It may be stated therefore that not only can fragmentation of chromosomes at the secondary constriction region increase the number of nucleoli in a species but it can also change the karyotype and increase the number of chromosomes. Differentiation in the body of the chromosomes, leading to the origin of supernumerary constrictions, should therefore be looked upon as an evolutionary process and a precursor to the origin of new karyotypes and higher chromosome numbers in species.

(iii) *Variation of chromosome number in Cucurbitaceae and its cytogenetic interpretation*

From Table 1 it will be seen that the chromosome number in the family Cucurbitaceae varies widely not only in different genera but also within a genus. The lowest number found is $2n=14$ in *Cucumis sativus*, the highest being $2n=48$ in *Cucurbita maxima*. There is a distinct aneuploid series in this family which may be represented as follows: $n=7$, 10, 11, 13, 16, 20, 21, 22 and 24. It will be evident from the present investigation as well as from the table above that numerical polyploidy has not played any significant role in the evolution of the Cucurbitaceae. There is evidence however of allopolyploidy in different members of this family. For instance in *Cucumis sativus* and *Luffa aegyptiaca* there is distinct secondary association between bivalents in the pollen mother cell which, according to the theory of secondary association, indicates homology between some of the bivalents. In *Cucumis sativus*, the secondary association is $2(2)+1$, the secondary number being three, which indicates that the basic number in the genus is three and not seven, the latter number being derived from the former.

Further, taking *C. sativus* and *C. Melo* as examples, we find no indication of duplication of chromosomes in *C. Melo*. Besides, the specific and identifiable chromosomes *A*, *B*, *C* and *D* of *C. sativus* are absent in the chromosome complement of *C. Melo*. This at once indicates that the higher number of chromosomes in *C. Melo* has not been derived by numerical polyploidy. In addition, *C. sativus* and *Luffa aegyptiaca* do not show any multivalent formation during meiosis, which confirms that these species are not autopolyploids.

From the study of secondary association between bivalents we find that the basic numbers of chromosomes are as low as 3 and 5 in case of *Cucumis sativus* and *Luffa aegyptiaca* respectively. The chromosome number of some genera of Cucurbitaceae has not yet been determined, but it is not unlikely that a lower number than 7 will be found in genera like *Fevillea*, *Thaladiantha*, etc., which from the taxonomic point of view represent a more primitive condition than *Cucumis*, *Cucurbita*, *Luffa*, *Citrullus* and others.

The above evidence indicates that the increase of chromosome number in this family is primarily due to allopolyploidy, but the analysis of the karyotypes of *Cucumis sativus* and *C. Melo* shows that fragmentation of chromosomes at particular loci, the fragments subsequently behaving as independent chromosomes, has contributed to the increase of chromosome number in different members of the family. The above interpretation thus not only explains the normal pairing of chromosomes in the pollen mother cell and lack of multivalent formation during meiosis in species with higher chromosome numbers, but also accounts for the very infrequent occurrence of polyploid species on the one hand, and on the other the presence of distinct aneuploid numbers in a genus.

It should be pointed out here that the origin of extra chromosomes through fragmentation at the region of secondary constriction, as put forward in this paper, is quite different to that of the origin of iso-chromosomes (Darlington, 1940) by misdivision of the centromere. Further, the normal pairing behaviour and good fertility in all the species examined show the very stable and balanced nature of the chromosome complement, characters which are not expected with iso-chromosomes (Darlington & Janaki Ammal, 1945).

(iv) *Are sex chromosomes in higher plants nucleologenic?*

Recent observations on the behaviour of sex chromosomes in animals like insects and in plants like Bryophytes have shown that both *X*- and *Y*-chromosomes are nucleolar and also heteropycnotic. In *Drosophila* it is known that the *X*-chromosome has a secondary constriction and the *Y*-chromosome has a satellite, both of which are nucleolar (cf. Gates, 1939). An exception has been reported where the *X*-chromosome was found not to be nucleolar. This anomaly has, however, been interpreted by Kaufmann (1937) as due to a translocation between the *X* and an autosome.

Though sex chromosomes have been reported in dioecious flowering plants, the relation of *X*- and *Y*-chromosomes to nucleolar production is not yet known. Nakajima (1937) has reported the presence of sex chromosomes in *Trichosanthes japonica*, but the diagram which he gives in support of his observation is not convincing. Besides he has not shown the other bivalents in his diagram.

As already mentioned, *Coccinia indica* shows the presence of sex chromosomes of *XY* type. In the somatic tissue of the male plant a very big *X*-chromosome* is present, which is heteropycnotic and with a secondary constriction near the primary one. But in the female plant the *X*-chromosome is absent and the sex mechanism can only be determined from a study of meiosis.

According to Kumar & Deodikar (1940), the sex chromosome in *C. indica* is represented by *XX* in male and *XY* in female plants. Moreover, they have reported the diploid number of the species to be $2n=26$ instead of 24. The figures of the somatic chromosomes which the above authors have given in support of their contention indicate, however, very poor fixation of the chromosomes, and any interpretations drawn from such figures are liable to be erroneous. But possibly there may be chromosomal varieties of this species occurring in nature.

Thus it appears that sex chromosomes in higher plants, too, take part in the production of nucleoli. Whether this is a generalized condition or not can only be stated when more evidence is forthcoming.

Summary

Except *Benincasa cerifera*, the somatic chromosomes of all the species examined, namely *Cucumis sativus*, *C. Melo*, *Trichosanthes dioica* (♂ and ♀), *Luffa aegyptiaca*, *L. acutangula*, *Coccinia indica* (♂ and ♀), *Momordica charantia* and *Cucurbita maxima*, are very small. Most of the species are characterized by the presence of an unusually high number of satellites and secondary constrictions. *Cucumis sativus* ($2n=14$) has 8 conspicuous secondary constrictions and a pair of satellites. *Luffa aegyptiaca* ($2n=26$) has 12 distinct and 4 less distinct satellites. There are also another 4 chromosomes with satellite stalks alone at one end without sat.-heads. *Trichosanthes dioica* ($2n=22$) has 26 satellites in the somatic complement of the male plants whereas in the female plants only 20 satellites are distinguishable. Some chromosomes have satellites at either end.

Corresponding to the high number of satellites and secondary constrictions a high number of nucleoli is present in most of the species. The maximum number of nucleoli could only be determined in the dyad nuclei after Feulgen light-green staining.

While in *Cucumis sativus* there are 6 pairs of identifiable chromosomes, *A*, *B*, *C*, *D*, *E* and *F*, in *C. Melo* there are none of the peculiar chromosomes of *C. sativus*. The increase

* See note p. 245.

in chromosome number from $n=7$ in *C. sativus* to $n=12$ in *C. Melo* has been interpreted on the basis of fragmentation of some of the chromosomes of *C. sativus* at the region of unusually exaggerated secondary constrictions, the acentric fragments subsequently acquiring a new centromere. Additional evidence of this explanation has been found in the proportionate increase of the number of nucleoli in *C. Melo*, although there is only one pair of chromosomes with distinct satellites present in this species. Secondary association between bivalents, indicating secondary polyploid nature of the species, has been found in *C. sativus* and *L. aegyptiaca*. The maximum association in the two species is 2(3) + 1 and 3(3) + 2(2). The basic number in the two genera should be 3 and 5 respectively. The aneuploid number of chromosomes, so characteristic in the Cucurbitaceae, cannot be explained on the basis of auto-, allo- or secondary polyploidy.

Even assuming such low basic numbers, the high number of nucleoli and satellites present in different members of the family cannot be explained on the basis of any cytogenetical interpretations yet put forward. It is suggested that fragmentation of chromosomes at the secondary constrictions has increased the number of chromosomes, satellites and nucleoli in different members of the family. Fragmentation of chromosomes thus provides a physical basis of speciation. New secondary constrictions can differentiate out spontaneously in the body of the chromosomes. Origin of supernumerary constrictions should be looked upon as an evolutionary process and a precursor to the origin of new karyotypes and higher chromosome number in species.

Direct evidence of the nucleologenic property of sex chromosomes in higher plants has been found for the first time in male plants of *Coccinia indica* having well-marked sex chromosomes of *XY* type. The *X*-chromosome, which is three times bigger than the autosomes,* has a nucleolar constriction near the primary one. The *Y*-chromosome is probably also nucleologenic because the heteromorphic *XY* pair has often been found attached to the fused nucleolus during diakinesis.

In conclusion we wish to express our thanks to Prof. R. R. Gates for helpful suggestions and for kindly going through the manuscript. Our thanks are also due to Prof. S. P. Agharkar for giving us laboratory facilities to complete this work.

REFERENCES

AFIFY, A. (1944). *J. Genet.* **46**, 116–24.
BANERJEE, I. & DAS, M. (1937). *Indian J. Agric. Sci.* **7**, 497–510.
BERGER, C. A. (1941). *Symp. Quant. Biol. Cold Spr. Harb.* **9**, 19–22.
BHADURI, P. N. (1938). *J. R. Micr. Soc.* **58**, 120–4.
BHADURI, P. N. (1942*a*). *J. Genet.* **44**, 87–127.
BHADURI, P. N. (1942*b*). *J. Genet.* **44**, 73–85.
BHADURI, P. N. (1942*c*). *Ann. Bot., Lond.*, N.S. **6**, 229–44.
BHADURI, P. N. (1944). *J. R. Micr. Soc.* **63**, 91–120.
BÖNICKE, L. VON (1911). *Ber. dtsch. bot. Ges.* **29**, 59–65.
CASTETTER, E. F. (1926). *Amer. J. Bot.* **13**, 1–10.
DARLINGTON, C. D. (1940). *J. Genet.* **39**, 351–61.
DARLINGTON, C. D. & JANAKI AMMAL (1945). *Ann. Bot., Lond.*, N.S. **9**, 267–81.
DARLINGTON, C. D. & UPCOTT, M. B. (1941). *J. Genet.* **41**, 297–338.
DE MOL, W. E. (1928). *Cellule*, **38**, 7–64.
ERVIN, C. D. (1941). *Amer. J. Bot.* **28**, 113–24.
FERNANDES, A. (1936). *Bol. Soc. broteriana*, **11**, 87–146.

* See note p. 245.

GATES, R. R. (1939). *Nature, Lond.*, **144**, 794–5.
GATES, R. R. (1942). *Bot. Rev.* **8**, 337–409.
HEIMLICH, L. F. (1927). *Proc. Nat. Acad. Sci., Wash.*, **13**, 113–15.
HEITZ, E. (1931). *Planta*, **12**, 775–844.
IYENGAR, N. K. (1939). *Ann. Bot., Lond.*, N.S. **3**, 271–305.
KAUFMANN, B. P. (1937). *Cytologia, Fujii Jub. Vol.* pp. 1043–55.
KIRKWOOD, J. E. (1907). *Bull. Torrey Bot. Cl.* **34**, 221–42.
KOLLER, P. CH. (1932). *J. Genet.* **26**, 81–96.
KOZHUKHOW, S. A. (1925). *Bull. Appl. Bot. Pl. Breed.* **14**, 89–130.
KUMAR, L. S. S. & DEODIKAR, G. B. (1940). *Curr. Sci.* **9**, 128–30.
KURITA, MASAHIDE (1939). *Bot. Mag., Tokyo*, **53**, 505–10.
LUNDEGÅRDH, H. (1914). *Arch. Zellforsch.* **13**, 145–57.
MCCLINTOCK, B. (1931). *Res. Bull. Agric. Exp. Sta.* no. 163, pp. 1–30.
MCCLINTOCK, B. (1934). *Z. Zellforsch.* **21**, 294–328.
MCKAY, J. W. (1930). *Bot. Gaz.* **89**, 416–17.
MCKAY, J. W. (1931). *Univ. Calif. Publ. Bot.* **16**, 339–50.
MATSUURA, H. (1938). *Cytologia, Tokyo*, **9**, 55–77.
MENSINKAI, S. W. (1939). *J. R. Micr. Soc.* **59**, 82–112.
MEURMAN, O. (1925). *Comment. Biol., Helsingf.*, **2**, 1–104.
MORINAGA, T., FUKUSHIMA, E., KANO, T., MARUYAMA, Y. & YAMASAKI, Y. (1929). *Bot. Mag., Tokyo*, **43**, 589.
NAKAJIMA, G. (1937). *Cytologia, Fujii Jub. Vol.* pp. 282–92.
NANDI, H. K. (1936). *J. Genet.* **33**, 315–36.
NAVASCHIN, M. (1932). *Z. indukt. Abstamm.- u. VererbLehre*, **63**, 224–31.
PASSMORE, S. F. (1930). *Bot. Gaz.* **90**, 213–23.
PATHAK, G. N. (1940*a*). *Ann. Bot., Lond.*, N.S. **4**, 227–56.
PATHAK, G. N. (1940*b*). *J. Genet.* **39**, 437–67.
RAMANUJAM, S. (1938). *Ann. Bot., Lond.*, N.S. **2**, 107–25.
RAU, N. S. (1929). *J. Indian Bot. Soc.* **8**, 126.
RESENDE, F. (1937). *Planta*, **26**, 757–807.
SATO, D. (1936). *Cytologia, Tokyo*, **7**, 521–9.
SATO, D. (1937). *Cytologia, Fujii Jub. Vol.* pp. 80–95.
SATO, D. (1938). **9**, 203–42.
SATO, D. (1939). *Jap. J. Genet.* **15**, 36–43.
SATO, D. (1940). *Bot. Mag., Tokyo*, **54**, 66–72.
SCHULTZ, J., CASPARSSON, T. & AQUILONIUS, L. (1940). *Proc. Nat. Acad. Sci., Wash.*, **26**, 515–23.
SEMMENS, C. S. & BHADURI, P. N. (1939). *Stain Tech.* **14**, 1–5.
SIKKA, S. M. (1940*a*). *Ann. Bot., Lond.*, N.S. **4**, 427–64.
SIKKA, S. M. (1940*b*). *J. Genet.* **40**, 441–509.
SINOTO, Y. (1929). *Cytologia, Tokyo*, **1**, 109–91.
SINOTO, Y. (1938). *Cytologia, Tokyo*, **9**, 254–71.
SRINATH, K. V. (1940). *Cytologia, Tokyo*, **10**, 467–91.
STRASBURGER, E. (1910). *Jb. wiss. Bot.* **48**, 427–520.
SUGIMOTO, T. (1928). Cited from *Tabul. biol., Berl.*, **7**, 184.
SUTARIA, R. N. (1936). *J. Univ. Bombay*, **4**, 21.
TISCHLER, G. B. (1931). *Tabul. biol., Berl.*, **7**, 184.
WHITAKER, T. W. (1930). *Amer. J. Bot.* **17**, 1033–40.
WHITAKER, T. W. (1933). *Bot. Gaz.* **94**, 780–90.

CONTAMINATION OF SEED CROPS

I. INSECT POLLINATION*

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(With Eight Text-figures)

Introduction

It is widely recognized that many agricultural and horticultural crops which are propagated by seed are liable to show a steady deterioration in quality and yield. The seed-growers counteract this tendency to some extent by roguing (i.e. destruction of plants not true to varietal type) and constant re-selection, but they have also endeavoured to remove the causes of this deterioration. One cause which has received much attention is contamination, whether it be due to admixture of foreign seed at harvesting, or admixture of foreign pollen during flowering. The former can be dealt with in a relatively straightforward way, by attending to the effective cleaning of threshing machines, etc., between the handling of different varieties of the same species. Its elimination will also diminish the latter kind of contamination because odd plants of one variety in a field of another will be very efficient suppliers of foreign pollen. It will not, however, completely eliminate contamination by foreign pollen which can be transported over relatively large distances through the agencies of insects or wind, against which we cannot take absolute precautions. Before such precautions can be considered our knowledge of the way in which pollen contamination may arise must be increased.

In spite, however, of the absence of any detailed knowledge of this subject seed-growers have had to devise some method of control. This has usually consisted of the provision of large isolation distances between crops which are likely to cross-pollinate. Distances up to a mile or so are commonly recommended (Haskell, 1943), but though few empirical data have so far been published on the effects of isolation distance, such as are available (Bateman, 1946) are in sharp contrast to the distances recommended in the trade. In the paper referred to above, it has been shown that though seed-growers have apparently used excessive isolation distances, they concentrate their efforts on the prevention of contamination between very distinct varieties of crops, and have overlooked the serious dangers inherent in contamination between relatively similar varieties. This latter type of contamination, though hidden ('cryptic'), may have a larger effect on varietal standards as it can give rise to polygenic variation which cannot be readily rogued out.

It appears then that there is a need for some systematic work to determine under standard conditions what are the effects of isolation distance on various crops. These results should then be applied to all varieties whether the contamination involved were obvious or cryptic, subject to the recognition that isolation requirements of crops vary from one to another and that for the same crop they will vary according to the different purposes (élite, stock or commercial) for which the seed is grown (Bateman, 1946).

Much, however, of the data on contamination so far obtained has been produced under

* Adapted from part of a thesis accepted for the degree of Ph.D. in the University of London.

conditions too unstandardized for them to be of great practical value to the seed-grower. Attention has been focused on the isolating effect of distance whilst the effects of isolation in time, size and arrangement of the plots of the contaminant and contaminated varieties have been largely ignored. As will be seen below, the latter factors are equal to distance in their importance in determining contamination.

Another factor which might be expected to influence contamination in entomophilous plants is the relation between the number of pollinating insects and the supply of nectar. This has been shown by Butler (1943) to affect the method of working of insect pollinators. This is to some extent under the grower's control through adjustment of the total amount of crops in flower at once and of the number of hives in the neighbourhood. Similarly in anemophilous crops wind direction and speed will influence contamination and it can be partly controlled by altering the relative spatial positions of varieties and breaking the wind with walls or trees. The characteristic breeding system for each species, to which the most important contributory factor is the degree of natural self-fertilization (compounded of self-incompatibility, diœcy, monœcy, protandry and even the number of flowers open on a plant at one time) will also influence a crop's liability to contamination. This complex of factors is almost outside the grower's control.

In the work described in the following account an attempt has been made to obtain a more vigorous control than hitherto of contamination-determining factors, and thereby to achieve results more generally applicable to seed-growing practice.

This is not to say that valuable work has not already been undertaken on the effects of isolation distance on contamination. Such is the experiment of Crane & Mather (1943) using two radish varieties (the same as those used in the experiments described below) such that the hybrid could be distinguished from either parent, in which they showed that the relationship between contamination and distance was not a linear one. Where isolation distance was small the rate of decrease of contamination with a given increase in distance was greater than when the isolation distance was larger. They also demonstrated the effect of the mass of plants of one variety growing together on the extent of their contamination by another variety. For in one experiment, in which both varieties were laid out in square blocks with one side in common, the spaces between plants being 9 in., contamination was 0·02 at 15 ft. from the common side, while in an experiment in which a single row of plants of one variety at intervals of up to 24 ft. was strung out from a square plot of the other variety, contamination fell to 0·02 only at a distance of 150 ft.

Currence & Jenkins (1942) studied the effect of distance on contamination in the tomato. Though this crop is self-compatible, many American varieties do not self-pollinate automatically because the style protrudes beyond the cone of anthers. They are thus prone to contamination. Choosing two varieties such that the hybrids were obvious in the first generation, these authors designed an experiment with a central square of one variety and two stringers in opposite directions of the other variety. Contamination in the stringers decreased to a minimum at the maximum isolation distance studied, which was 72 ft.

Balls, Templeton, Brown & Kilain (1929) published results on contamination in cotton. These showed the expected decrease in contamination with increasing isolation distance, but the senior author was undecided whether the contamination was inversely proportional in the distance or to an exponential function of the distance. A marked seasonal fluctuation to the extent of contamination was correlated with seasonal variation in the numbers and

kinds of insect visitors. Under Egyptian conditions, hive-bees visited the flowers between epicalyx and corolla thus avoiding pollination. The bee mostly responsible for contamination was a species of solitary bee, and most of the contamination was brought about during a short period when this bee was very active.

There appears to be only one instance in which seed-growing practice has assumed contamination to vary in any definite way with isolation distance. This is contained in the regulations of the Minnesota Crop Improvement Association as reported by Hayes & Immer (1942). It concerns the production of hybrid corn. For a hybridizing plot of size 5 acres, 40 rods (approx. 200 m.) is the minimum isolation distance. This minimum may be reduced, however, if extra border rows of the male parent are planted round the plot in such a way that a reduction in isolation distance of 2½ rods (12·6 m.) can be compensated for by one additional border row. For example, if the isolation distance is 10 rods, twelve border rows must be planted. That the actual effect of isolation distance is not so simply equivalent to border rows is implied by the proviso that border rows may only be substituted for distance when the danger of contamination comes from a variety with the same seed colour as the female parent of the hybrid. Where the intended parents of the hybrid corn had the same seed colour and the F_1 showed no complementary gene action for colour this arrangement would usually involve cryptic contamination.

In the experiments on contamination here presented, the choice of the pair of varieties and which of the pair is to be used as the seed parent of the test progenies, has been made so as to facilitate the early identification of contamination. That is, contamination has always been of an obvious kind, it being assumed that the rules governing cryptic contamination are the same. As interest centres on low frequencies of contamination it is necessary to use large progenies. Earliness in the stage at which contamination is identifiable is important in enabling larger progenies to be grown in the same space and scored in a shorter time. Finally, the crops chosen are those in which contamination is liable to be high, as these are most suitable for work on the external factors concerned with contamination. Such is the case with the following four crops: turnip and radish; both self-incompatible insect-pollinated crops: beet; self-incompatible and generally wind-pollinated, though partially insect-pollinated: and maize; wind-pollinated and self-compatible, but being monœcious and strongly protandrous, a naturally outbreeding crop. The crops beet and maize are dealt with in a later report.

The experiments were designed to study independently the effects on contamination of the distance of the tested variety from contaminating plants and the number of plants of the tested variety growing together. This number is later referred to for simplicity as the mass. The mass does not have a direct effect on the amount of non-contaminant pollen available, as the contribution of pollen from any one plant to the stigmatic surface of another will vary with the distance between them. One can expect, therefore, that the same mass will give variable protection against contamination according to the distribution and density of the plants and the position within the plot of the plant examined for contamination.

It was thought desirable to study the effect of distance over the maximum possible range. For this purpose a series of experiments was arranged, each suitable for the study of the distance effect over a different range. The standard plan consisted of a central plot of the contaminant variety from which extended in various directions arms of varying width and spacing. For such arrangements the problem arises as to what is the zero

isolation distance. Since preliminary results suggested that the nearest plants made the biggest pollen contribution, isolation distance was measured from the nearest edge of the contaminant block. At the same time experiments were also designed in which the contaminant was concentrated at a point in the centre of a block of the other variety. Here there could be only one point from which to measure isolation distance. This design also enabled one to vary the density of planting while maintaining the isolation distances and the relative amounts of the two varieties constant.

In an experiment with arms of varying numbers of rows, comparison of the progenies of plants in the same row of the same arm gave the distance effect, and comparison of the progenies of plants in different rows at the same distance gave the mass effect. Such an arrangement is well suited to statistical analysis, permitting independent tests of significance of the various effects.

The dependent variable in these analyses is the proportion of hybrid seedlings to the total progeny, the error variance of a proportion varying with its magnitude according to the general formula, $V_p = pq/n$. As there is a general decrease in this proportion with increasing distance the error variance will always be higher for short distances than for long ones, and the error variance estimated for the whole experiment will be too low for tests of significance at short distances and too high for tests of significance at long distances. But if we transform the frequency of contamination F into an angle ϕ, such that $F = \sin^2 \phi$ (Snedecor, 1946)* the new variable has an error variance independent of its magnitude and depending only on the size of the sample, which shows no trend with distance. The variance of ϕ, measured in degrees, is $820{\cdot}7/n$. Where n, the size of the sample, is not constant, to calculate the theoretical error variance of a number of observations the harmonic mean of the individual sample sizes is used in place of n. The ratio of the sum of squares of error obtained from the analysis of variance to the theoretical error variance gives χ^2 with the same number of degrees of freedom as the error sum of squares. A significantly high χ^2 would show that factors other than sample size were contributory to the error variance.

In the following account, the variety being tested for the contribution of its pollen to the seed of the other variety is referred to as the 'contaminant' and the proportion of progeny produced by hybridization between the two varieties is referred to as the 'contamination' and symbolized by F.

Data for out-breeding insect-pollinated crops

In this section the two species studied were turnip and radish. The varieties and hybrids used in the experiments are listed below with their diagnostic features and the stage at which detectable.

Crop	Seed parent		Contaminant		Hybrid	
	Name	Appearance	Name	Appearance	Appearance	Stage at which identifiable
Turnip	White Milan	White swollen root	Red-Top Milan	Red top to swollen root	Red top to swollen root	Red pigment shows in young leaves and at leaf bases
Radish	Scarlet Globe	Deep red globular root	Icicle	White long root	Purple longish root	Colour evident in unswollen hypocotyls

* Fisher and Yates *Statistical Tables* (1938) give a less detailed table for angular transformations.

As it has a bearing on the subject of this study it is worthy of note that there appeared to be some contamination in the seeds used for this experiment which were obtained from seedsmen. Seed of Scarlet Globe Radish produced some plants which were apparently identical with the F_1 between that variety and Icicle. In 1945 commercial seed of Scarlet Globe was scored for the frequency of purple rogues of this type, which was found to be 10 out of 1355 or rather less than 1 %. In the author's opinion this is a reasonable amount to expect in commercial seed. However, the frequency of purples only represents a small proportion of the total variability in the variety, as there was a considerable range in the intensity and distribution of the red pigment in the hypocotyl and there was also variation in hypocotyl shape. One might expect that plants of the Icicle variety (which is not a very popular one) would only be responsible for a small proportion of the contamination occurring in Scarlet Globe commercial seed. Amongst Icicle seedlings were found plants with yellow and black hypocotyls.

Seed of White Milan Turnip produced purple-topped plants which on the first occasion, because no roguing had been carried out, were a source of confusion in interpreting the results. Seedlings of White Milan from seed sown in the autumn of 1943 were rogued of all plants showing any anthocyanin in the hypocotyl. This eliminated all purple tops from the experimental plants. A high proportion of green-topped plants remained, though these did not interfere with the experiment.

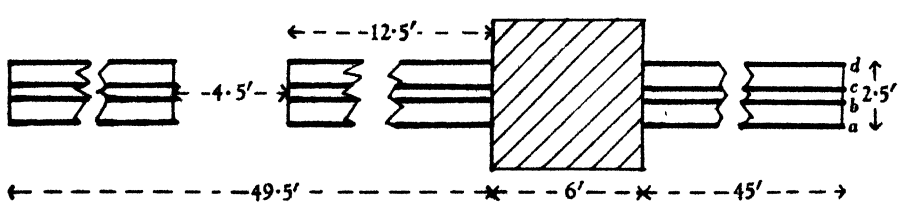

Fig. 1. Plan of Exp. 1. Hatched square represents Red-Top Milan plot from which extend two stringers of White Milan. Heavy black lines represent the four sampled rows.

Experiment 1. This consisted of a central square plot of Red-Top Milan with two arms of White Milan extending from it to east and west. Details of arrangement and dimensions are shown in Fig. 1. Seeds were sown in the autumn of 1942 and the plants planted out in the spring of 1943. Seed was harvested in June 1943. The plants sampled were at various distances from the contaminant plot, in the innermost and outermost lengthwise rows of both arms. The plants in each row were numbered 1–90 beginning from the contaminant plot. As the western arm was interrupted by a path 4 ft. 6 in. wide between plants numbered 25 and 26, each plant in the eastern arm of number greater than 25 had two corresponding plants in the western arm, one of the same number, that is with the same number of plants between it and the contaminant, and one of the same distance from the contaminant, the latter being 4 ft. 6 in. nearer the contaminant than the former. The sampled plants in the eastern arm were numbered 1, 2, 3, 4, 9, 16, 25, 36, 49, 64, 81, 90. Each had a suffix *a*, *b*, *c* or *d* according to the row from which it came; *a* and *d* being the outermost rows, and *b* and *c* the innermost. In the western arm plants corresponding to those in the eastern arm were sampled. That means two plants for every one in the eastern arm numbered 36 and higher with the exception of plants 90. The only harvested plants in the western arm corresponding to these were those numbered 90. Each sampled plant had five siliquae harvested from different heights on the main axis of the inflorescence.

1A and 1B were the first and second siliquae respectively and 2, 3 and 4 were equally spaced up the remainder of the axis. Siliquae of the same number from different plants did not necessarily coincide in flowering time, but as a whole, siliquae from position 3 had bloomed earlier than siliquae from position 4. As the average number of seeds per siliqua was only about twelve, all siliquae from one plant were pooled in the eastern arm, and all siliquae of the same height from plants of the same number, were pooled in the western arm. In this way, both arms yielded information on the effect of isolation distance on contamination, whilst the eastern arm showed whether there was any effect of row and the western arm whether there was any effect of time of flowering on contamination.

One distance in the western arm (40·5 ft.) has been omitted from the analysis of variance. As will be seen in Table 1, this had an abnormally high value for F, viz. 0·109. During

Table 1

Row no.	Distance from contaminant in ft.	West arm Position of siliqua 1 A	1 B	2	3	4	Mean	East arm Row *a*	*c*	*d*	*f*	Mean
1	0·5	0·200	0·313	0·564	0·607	0·176	0·372	0·486	0·417	0·071	0·492	0·366
2	1	0·333	0·578	0·406	0·500	0·263	0·416	0·059	0·093	0·073	0·387	0·133
3	1·5	0·460	0·259	0·326	0·391	0·385	0·364	0·340	0·064	0·275	0·382	0·265
4	2	0·231	0·086	0·179	0·364	0·211	0·214	0·333	0·045	0·128	0·406	0·228
9	4·5	0·063	0·094	0·135	0·107	0·188	0·117	0·316	0·300	0·069	0·040	0·181
16	8	0·000	0·019	0·029	0·025	0·037	0·022	0·000	0·029	0·016	0·184	0·057
25	12·5	0·029	0·024	0·027	0·054	0·000	0·027	0·000	0·129	0·000	0·000	0·032
—	18	0·000	0·031	0·000	0·000	0·000	0·006	0·023	0·032	0·000	0·000	0·014
36	—	0·000	0·000	0·000	0·000	0·000	0·000					
—	24·5	0·000	0·107	0·000	0·000	0·000	0·021	0·000	0·000	0·000	0·000	0·000
49	—	0·000	0·000	0·000	0·000	0·000	0·000					
—	32	0·000	0·000	0·000	0·077	0·000	0·015	0·143	0·028	0·000	0·000	0·043
64	—	0·000	0·000	0·000	0·000	0·000	0·000					
—	40·5	0·257	0·094	0·129	0·067	0·000	0·109	0·000	0·000	0·000	0·000	0·000
81	—	0·025	0·000	0·000	0·000	0·000	0·005					
—	45	—	—	—	—	—	—	0·000	0·000	0·000	0·000	0·000
90	—	0·000	0·000	0·000	0·080	0·000	0·016					

Figures for proportion of contamination of White Milan Turnips by Red-Top Milan. Those in the west arm are the result of pooling the seed from corresponding siliquae in the rows represented in the east arm. Those in the east arm are the results of pooling the seed for the five siliquae represented in the west arm for each plant sampled. At distances over 12·5 ft. from the contaminant the west arm has two samples for every one in the east arm, as shown above.

flowering a survey had been made of plants in the White Milan Arm showing abnormally high anthocyanin content. Only one of these suspect plants happened to be sampled for analysis. This was 72*a*, one of the plants sampled at distance 40·5 ft. It seems justifiable to assume that in this case the high proportion of purple plants was due to one plant being heterozygous for the contaminating gene. Consequently, it was discarded for the analysis.

The analysis of variance in Table 2 leads to the following conclusions: (i) The distance effect is highly significant. This is not surprising. (ii) Position of siliqua (i.e. time of flowering) and (iii) Row, are on the borders of significance. If these effects did prove to have a real foundation they would signify that contamination is highest during the middle of the flowering period, and that it is higher in outermost rows than in innermost rows, which means that it is higher when plants are bordered on only three sides by their own variety than when they are surrounded by them. The latter is borne out by later experiments. As to the former possible effect, since both varieties corresponded very closely in flowering period, significance would imply variation in some external factor such as the relative

abundance of the pollinators, which has been shown by Balls *et al.* (1929) to be significant in cotton.

No attempt is made here to explain the results in terms of insect activity. This will be dealt with in a later paper.

Table 2

	Sum of squares	N	Mean square	Variance ratio	Probability
		West arm			
Position (i.e. time)	31695·42	4	7923·85	2·32	>0·05
Distance	1604536·35	14	114609·74	33·61	Very small
Error	190984·58	56	3410·44	—	—
Total	1827216·35	74			
		East arm			
Distance	745336·23	11	67757·84	7·80	<0·001
Inner and outer rows	28081·69	1	28081·69	3·23	>0·05
Other row effects	16308·37	2	8154·18	—	—
Error	286624·19	33	8685·58	—	—
Total	1076350·48	47			

Analysis of variance of results in Table 1 after they had been submitted to the angular transformation.

The error variance for plants of number over 25 is too great in comparison with contamination for us to judge whether the number of intervening plants between the sampled plant and contaminant plot or the isolation distance is the more important factor in determining the amount of contamination.

Experiment 2. The maximum isolation distance encountered in the above experiment was 49·5 ft. In order to obtain information on the effects on contamination of greater distances, two trials were planned for 1944; one with radish and one with turnip. The designs of both were essentially the same. The contaminant blocks consisted respectively of 121 plants of Icicle Radish and 441 plants of Red-Top Milan Turnip, both being 10 ft. square. The two blocks were side by side. From these in two directions, east and south, plants of Scarlet Globe and White Milan were strung out in straight lines at 20 ft. intervals, up to a distance of 580 ft. in the east stringer and 480 ft. in the south stringer. The experiments were designed in this way to obtain a measurable amount of contamination at all distances, whilst it was hoped that by increasing the general level of contamination the nature of the effect of distance would remain unaltered. All plants in the stringers were harvested.

Considering the radish trial first, thirty capsules, where present, were taken from all parts of the inflorescence. It was considered desirable to keep the factor n, in the expression $V_p = pq/n$ as constant as possible and at the same time as large as possible. So counts were made of the number of seeds harvested in some representative samples. From these it was decided that 150 was the progeny number giving the best combination of constancy and large size. Consequently 150 seeds, when available, were sown from each Scarlet Globe plant. The results are shown in Table 3 and shown graphically in Fig. 2. It is immediately clear that in spite of the much greater distances involved and the different arrangement of the plants the effect of distance on contamination is essentially similar to that in the 1943 turnip trial.

But closer examination shows a new feature in the results. Beyond plant 8 (160 ft.) in either arm, there does not appear to be any further reduction in contamination, as can be seen in Fig. 2. As the error variation is high in comparison with average contamination

Table 3

Plant no.	Distance in ft.	Radish contamination: East stringer	Radish contamination: South stringer	Turnip contamination both stringers pooled
1	20	0·573	0·642	0·393
2	40	0·434	0·143	0·330
3	60	0·156	0·053	0·258
4	80	0·169	0·106	0·167
5	100	—	0·055	0·170
6	120	0·147	0·049	0·125
7	140	0·084	0·037	0·087
8	160	0·017	0·000	0·030
9	180	0·035	0·024	0·036
10	200	0·000	0·000	0·000
11	220	0·000	0·035	0·086
12	240	0·010	0·017	0·000
13	260	0·000	0·021	0·000
14	280	0·018	0·000	0·000
15	300	0·042	0·007	0·000
16	320	0·000	0·021	0·000
17	340	0·007	0·007	0·000
18	360	0·000	0·000	0·000
19	380	0·008	0·000	0·000
20	400	0·015	0·008	0·000
21	420	0·011	—	0·018
22	440	0·000	0·019	0·105
23	460	0·000	0·025	0·000
24	480	0·000	0·087	0·045
25	500	0·008	—	0·000
26	520	0·021	—	0·000
27	540	—	—	0·000
28	560	0·000	—	0·000
29	580	0·008	—	0·000

Results of long-distance radish trial (two arms given separately), and long-distance turnip trial (result of pooling both arms).

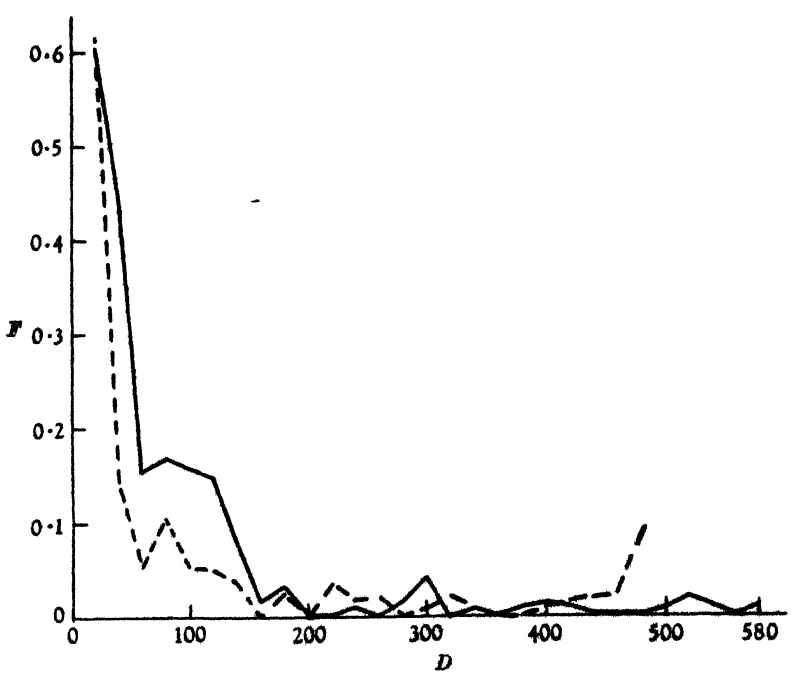

Fig. 2 (Exp. 2). Graph showing effect of distance, D, measured in feet, on contamination, F. ——, east stringer - - - -, south stringer.

at these distances, it is unwise to draw any conclusions from the appearance of the graph. But we can test the constancy of contamination beyond plant 8 by dividing all the plants concerned into two equal groups, proximal and distal, and summing all the frequencies within each group. We can submit the resulting figures to a χ^2 test.

In the west stringer	Purple	Red	Total	χ^2	Probability
Total for plants 8–18	14	1138	1152	1·40	0·2 to 0·3
Total for plants 19–29	9	1210	1219		
In the south stringer					
Total for plants 8–15	13	794	807	0·23	0·5 to 0·7
Total for plants 16–24	21	1082	1103		

The χ^2 has one degree of freedom. The evidence does not suggest any reduction in contamination from the proximal groups to the distal groups.

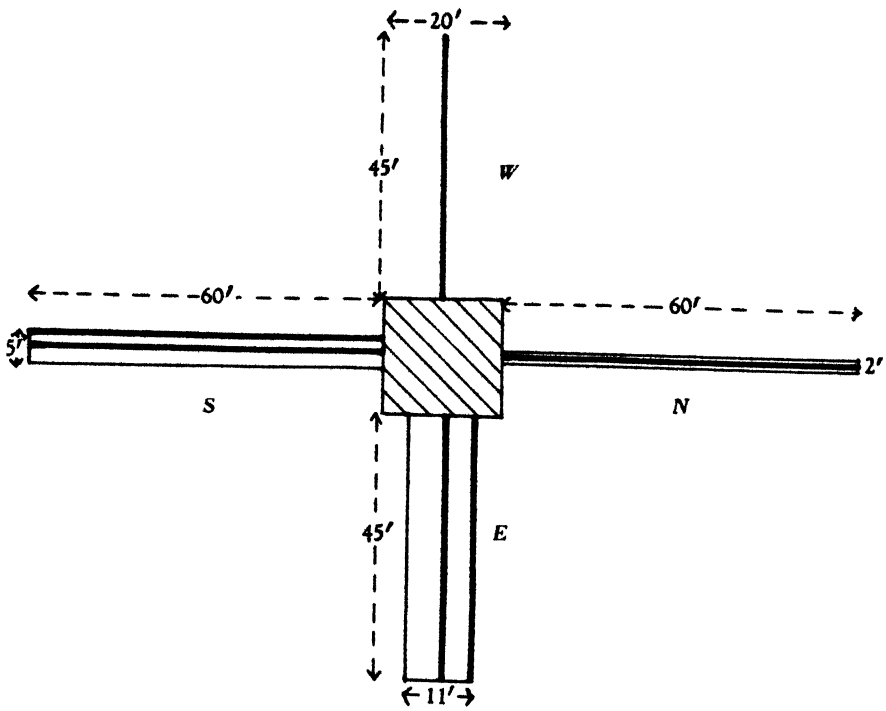

Fig. 3. Plan of Exp. 3. Hatched square represents Icicle plot from which extend four arms of Scarlet Globe. Heavy black lines represent the sampled rows.

In the parallel turnip experiment, depredations by birds forced a premature harvest. Consequently, seed samples were small and of poor germination (average family size 26). Owing to the larger error variance the results of the south and east stringers are pooled and F calculated from the total progenies. These values are shown in Table 3. Though of little value in themselves they serve to confirm the radish results.

Experiment 3. A sub-significant suggestion in the 1943 turnip experiment that the outer rows of a stringer had higher contamination than the inner rows, led to the design and execution of an experiment in 1944, using radish, to test the possible effect of mass of plants on contamination. The lay-out, shown in Fig. 3, consists of a central contaminant block of Icicle with four arms of Scarlet Globe comprising 1, 3, 6 and 12 rows running in W, N, S and E directions respectively. Further data are obtained on the effects of distance, and mass can be studied in two ways; by comparing plants in central rows of each arm,

and by comparing plants in innermost (*Ei*, *Si*) and outermost (*Eo*, *So*) rows of the two widest arms. The latter method further provides an opportunity for an estimate of any interaction between mass and distance.

Though the Scarlet Globe sowings were rogued for purple-coloured roots, the process cannot have been complete. For at harvest it was discovered that one plant whose seed was being collected had a purple root (*So* 15). Out of its progeny of 141, 74 were purple, a typical back-cross ratio. Furthermore, as one might expect from the more intense

Table 4

Distance in ft. from contaminant	*W*	*N*	*Si*	*So*	*Ei*	*Eo*
1	0·607	0·586	0·419	0·500	0·278	0·378
2	0·584	0·331	0·154	0·218	0·138	0·250
3	0·250	0·183	0·168	0·321	0·159	0·158
4	0·359	0·275	0·143	0·257	no sample	0·171
5	0·248	0·086	0·132	0·237	0·055	0·089
10	0·068	0·048	0·076	0·074	0·013	0·014
15	0·066	0·007	0·000	*	0·000	0·029
20	0·031	0·029	0·000	0·017	0·031	0·000
25	0·035	0·000	0·005	0·015	*	0·008
30	0·016	0·007	0·000	0·014	0·000	0·000
35	0·014	0·007	0·000	0·000	0·000	0·022
40	0·013	0·007	0·000	0·015	0·000	0·000
45	0·061	0·010	0·014	0·000	0·000	0·000
50	—	0·007	0·000	0·007	—	—
55	—	0·000	0·000	0·009	—	—
60	—	0·000	*	0·007	—	—

Values for contamination in Exp. 3. An * represents a value discarded because there was evidence of contamination brought forward from previous generations (see text).

pigmentation in Scarlet Globe than in the F_1 hybrid, these back-cross purples were more intensely purple than the F_1. Apart from this abnormal family which was discarded from the analysis there were two other plants, *Ei* 25 and *Si* 60 which contained amongst their progeny intensely purple seedlings and showed abnormally high apparent contamination. It was assumed that this also was due to contamination in a previous generation and these progenies were also discarded from the analysis.

Table 5

	Sum of squares	*N*	Mean square	Variance ratio	Probability
		Using series *W*, *N*, *Si* and *Ei*			
Mass	868·128	3	289·376	15·70	<0·001
Distance	7687·326	10	768·733	41·67	Very small
Error	553·465	30	18·449	—	—
Total	9108·919	43			
		Using series *Si*, *So*, *Ei* and *Eo*			
Mass	136·900	1	136·900	12·73	<0·01
Position	85·849	1	85·849	7·98	<0·05
Distance	6520·979	9	724·553	67·38	<0·001
$M \times P$	15·876	1	15·876	1·48	>0·2
$M \times D$	233·970	9	25·997	2·42	0·2–0·05
$P \times D$	101·810	9	11·312	1·05	High
Error	96·775	9	10·753	—	—
Total	7192·159	39			

Analysis of variance for the angular transformation of the result shown in Table 4.

150 seeds, where available, were sown from each plant sampled. These were at distances from the contaminant of 1, 2, 3, 4, 5, 10 ft. and thence every 5 ft. to the end of the arm.

Table 4 shows the proportions of contamination in plants at varying distances and positions in each of the four arms. Fig. 4 shows graphically the results for the central row of each arm. The analyses of variance in Table 5 are carried out on the angular transformation, those distances not represented in all four rows being omitted.

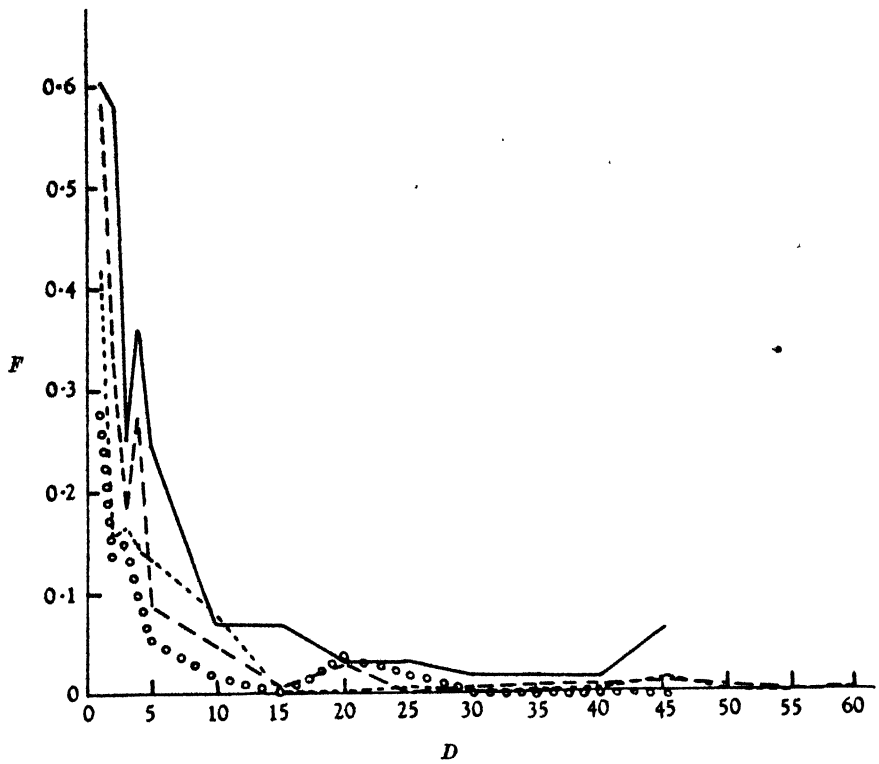

Fig. 4 (Exp. 3). Graph showing effects of distance in feet, D, and width of arm, on contamination, F. ——, one-row arm, W; – – – –, three-row arm, N; · · · ·, six-row arm, Si; o o o o, twelve-row arm, Ei.

In the first analysis (for the four centre rows only) the error mean square (18·449) includes any mass-distance interaction there may be. In the second analysis (for two rows of each of the largest arms) this interaction (M.S. = 25·997) is separable from the error mean square (10·753) and does not quite reach the 5 % level of significance.

As the theoretical error variance of ϕ is based solely on the size of the sample, it can be estimated from the data (see introduction). The χ^2 derived by means of this method is a test of whether factors other than sample size make a significant contribution to the error variance estimated from the data.

In the first analysis of variance the theoretical error variance is 8·8115;

$$\chi^2_{(30)} = \frac{553{\cdot}465}{8{\cdot}8115} = 62{\cdot}81;\ P = 0{\cdot}001.$$

In the second analysis the theoretical error variance is 6·3389;

$$\chi^2_{(9)} = \frac{96{\cdot}775}{6{\cdot}3389} = 15{\cdot}27;\ P = 0{\cdot}1 - 0{\cdot}05.$$

From the second analysis we may conclude that sample size can account for the whole error variance. In the first analysis this is not so. As the first error variance includes the

mass-distance interaction while the second does not, the mass-distance interaction here being separable and not quite significant, we are justified in assuming that the high χ^2 obtained from the first analysis is due to the mass-distance interaction.

The effects of mass and of the mass-distance interaction can be seen by grouping the families of each arm into two groups, distal and proximal, and calculating the mean value of F for each group. The proximal value is calculated from plants at distances 1, 2, 3, 5 and 10 ft.: the distal value from plants at distances of 20, 30, 35, 40 and 45 ft. The missing distances are those not represented in all rows sampled. The mean values are shown in Table 6 and graphically in Fig. 5. In the latter the logarithm of F has been used so that the relative effects of mass can be compared in the distal and proximal portions.

Table 6

Arm	No. of rows	Proximal	Distal	Proximal/Distal
W	1	0·3514	0·0270	13·01
N	3	0·2468	0·0120	20·57
S	6	0·2299	0·0060	38·32
E	12	0·1532	0·0053	28·91

Exp. 3. The mean contamination in the proximal and distal parts of the four arms (inner and outer rows of *S* and *E* averaged) to show the influence of mass of arm on the relative effect of increased distance from contaminant.

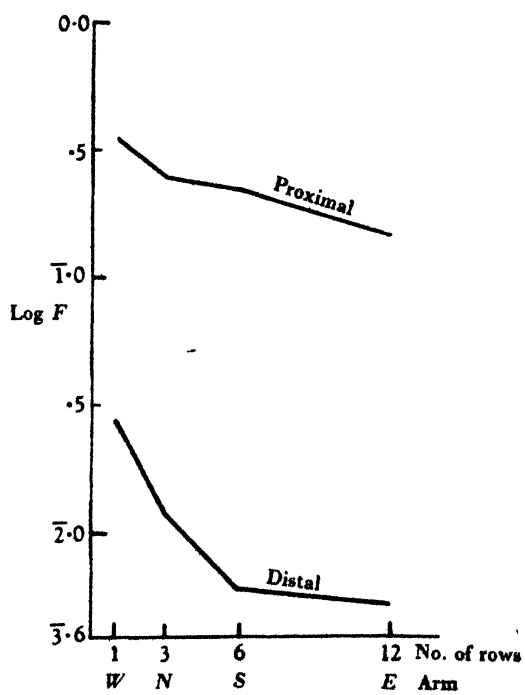

Fig. 5 (Exp. 3). Graph showing effect of isolation distance on the relative decrease in contamination with increasing width of arm. Log F is plotted against row-number for the proximal and distal parts of each arm separately. Proximal is up to 10 ft. Distal is 15 ft. to 45 ft.

It is clear that, generally speaking, increasing the number of rows decreases contamination. In the proximal part of the arms this effect is maintained within the limits of the experiment, from one row to twelve. The relation appears to be described in the graph by

a straight line. In other words, the *relative* rate of decrease in contamination produced by the addition of one row remains constant, though of course the *absolute* rate will decrease with increasing numbers of rows. This effect is similar to that of unit increase in isolation distance on absolute contamination, which has been observed in all experiments so far for distances of less than 160 ft.

In the distal part of the arms, however, the relative decrease in contamination per row increase is not constant. It is high where the number of rows is small, higher in fact than the rate in the proximal part, but beyond three rows, increase in row number has a very slight effect and beyond six rows the effect is negligible. We can conclude, that, where the isolation distance is greater than a certain small amount (equivalent to 15 ft. under the conditions of the experiment) and where the mass is above a certain minimum (equivalent to six rows in this experiment), variation in mass has a negligible effect on contamination.

To give a practical application of this conclusion we could state that in the case of a field of a crop being grown for seed at a moderate distance from a contaminant variety, provided the outer rows were discarded, the amount of contamination would be independent of the size of the field.

One possible criticism of the validity of the result of the above experiment is that there may be interference by three of the arms of Scarlet Globe with the results in the fourth. The importance of such interference can be assessed by reference to the experiment.

The maximum interference by one arm with the result of another would be expected in the effect of the twelve-row arm (E) on the one-row arm (W). Let us consider plant $W1$, whose shortest distance from E is 22 ft. At 22 ft. from the contaminant plot, arm W registers a contamination of about 0·03. The contaminant plot has a frontage of twenty-one plants and a total of 441 plants. The arm E has a frontage of twelve plants and a total of 420 plants. We can assume, therefore, that 0·03 is a high estimate of the proportion of seed set on plant $W1$ due to pollen from arm E. By removing arm E we should expect the contamination of plant $W1$ to change from 0·607 as observed to $\frac{0{\cdot}607}{1-0{\cdot}03}=0{\cdot}626$. At 41 ft. from the contaminant, contamination in arm W is about 0·02. By a similar argument removal of arm E would increase the contamination of plant $W20$ from 0·031 to $\frac{0{\cdot}031}{1-0{\cdot}02}=0{\cdot}0316$. We conclude that the effect of arm E is to reduce the contamination in arm W by approximately the same small proportion throughout. The effects of arm E on arms N and S and of other arms on one another would in all cases be rather less. Interference between arms is not, therefore, such as to invalidate the conclusions.

Experiment 4. In all the experiments so far described contamination has been due to a large plot of the contaminating variety. This has been necessary to obtain measurable amounts over the distances used. It is difficult with such a design to know from what point to measure the isolation distance. A superficial examination of the results shows that plants in the immediate vicinity of a given plant are the most important in effecting its fertilization. Thus for plants near to a contaminant plot the isolation distance should be measured from a point near to the nearest edge of the plot. At greater distances it does not matter so much from which point the distance is measured. This difficulty in finding the zero isolation distance can be overcome if necessary by using $(D+\delta)$ for the isolation distance where D is the distance from the nearest edge of the contaminant plot and δ is a correction which may be estimated in some cases from the regression of F on $(D+\delta)$.

This is not reliable when the form of the regression is not known, as the estimated value of δ will vary with the regression used.

So in 1945 an experiment was carried out in which contamination was due to what was in effect a point source, i.e. a small number of plants crowded together. At the same time the possible effects of density of planting were investigated. Four densities were used, each represented by two square plots. Each square consisted of Scarlet Globe Radish plants with a number of Icicle plants at the centre proportional to the density of the plot, so that the ratio of Scarlet Globe to Icicle plants was constant. The internal arrangements of the squares at the four densities are shown in Fig. 6. The experiment was in duplicate, the squares being designated as *WA*, *WB*, *WC*, *WD*, in the western series and *EA*, *EB*, *EC* and *ED* in the eastern replication.

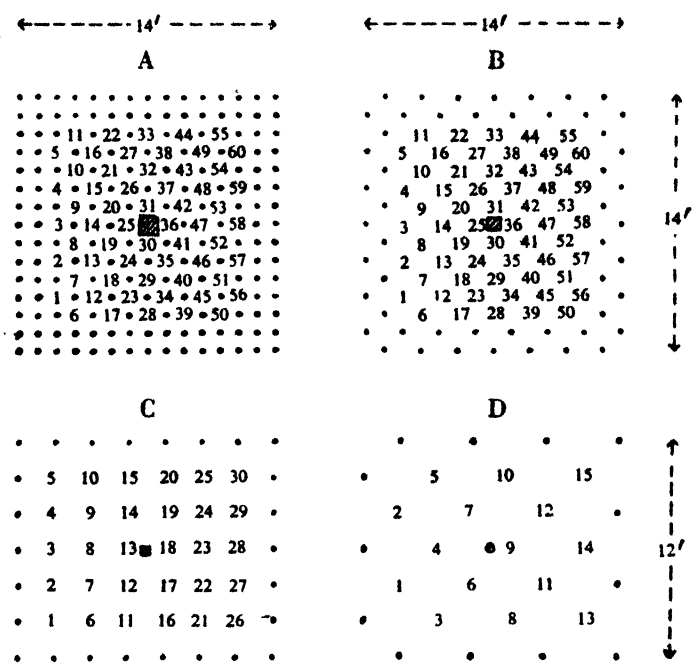

Fig. 6. Plan of Exp. 4. The central hatched area represents 8, 4, 2 and 1 Icicle plants in squares A, B, C, D respectively. All other plants are Scarlet Globe. All sampled plants are numbered, others being represented by a dot each. The squares are not shown in their relative positions. In fact, they were widely separated.

Density was varied in the anticipation that it would affect the extent of distribution of contamination. For, according to Butler (1943), the less the amount of nectar available in a given area for a constant number of working bees the further the bees would travel during foraging. This would lead one to expect that the least dense plots would show the slowest rate of decrease in contamination with increasing distance.

As the Icicle variety is later in flowering than Scarlet Globe, if the whole natural seed set on Scarlet Globe were used to estimate contamination the results would be diluted by a factor depending on the amount of seed set before the Icicle flowered, and to a varying extent for each plant sampled according to the time of onset of its own flowering. To eliminate this source of error, as the group of Icicle plants in each square came into flower all open flowers and capsules from the Scarlet Globe plants in the square were removed. *EA*, *EB*, *EC*, *ED*, *WB* and *WC* were treated in this manner on 30 May. Similarly with

WD on 7 June. In *WA* the Icicle plants were so late that the Scarlet Globe had almost completed flowering, so the contamination was negligible and the plot was valueless for the purpose of the experiment.

There was much depredation of the young capsules by birds. This was particularly marked for squares *EC* and *ED*. As all the outer (later) capsules were removed in these squares the remaining seed would not form a representative sample of the entire seed test.

As there are less plants per distance sampled in the sparser squares, increased error variance in these due to small sample size was avoided by adjusting the size of the sample of seed taken from each plant, so that eight times the amount of seed per plant was harvested from *ED* than from *EA*. There is another source of increased error variance in the sparser squares which was not avoided in the experiment; that due to variation between seed of plants in the same square and at the same isolation distance.

In Table 7 are shown the results of all sampled squares with the plants arranged according to distances. The same results are shown graphically in Fig. 7. The sample progenies

Table 7. *Summarizing contaminations in all the radish density squares* (*Exp.* 4)

Block	Distance in ft.	0·7	1·6	2·1	2·5	2·9	3·5	3·8	4·3	4·5	4·7	4·9	5·1	5·7	6·4
EA	Progeny size	423	900	639	1061	1051	1052	1028	1024	894	816	397	872	849	424
	F	0·111	0·079	0·063	0·055	0·029	0·011	0·015	0·008	0·006	0·013	0·008	0·010	0·006	0·002
	Distance in ft.	1·0		2·2		3·0	3·6		4·1			5·0	5·4		6·4
EB	Progeny size	624		1927		1051	2285		1855			2221	1835		1956
	F	0·223		0·120		0·135	0·047		0·047			0·028	0·026		0·017
EC	Progeny size	705		1070		1033	643		1873			1481	996		660
	F	0·027		0·058		0·105	0·033		0·021			0·021	0·011		0·018
ED	Progeny size	168		275		—	65		8			416	343		46
	F	0·190		0·138		—	0·062		0·250			0·034	0·015		0·022
WB	Progeny size	498		1639		1100	1631		1788			3085	693		1302
	F	0·227		0·103		0·069	0·044		0·026			0·012	0·012		0·011
WD	Progeny size	88		1752		950	464		1254			1813	1463		1235
	F	0·011		0·046		0·080	0·011		0·013			0·009	0·005		0·003
	Distance in ft.	1·4				3·2			4·2			5·1	5·8		7·1
WC	Progeny size	2210				4188			1810			3151	4071		2057
	F	0·119				0·053			0·017			0·016	0·009		0·007

The progeny sizes for each distance are the sums of the progenies of all plants at that distance.

from the outermost rows of squares are not included in this table and figure because in these rows the density is always less than in the rest of the square. Owing to slight errors in placing the plants the distances of sampled plants from the contaminant group are not the same in *EA* and *WC* as in the other squares. These slight differences in planting do not appear to affect the main results.

In Fig. 7 the squares *EA*, *EB*, *WB* and *WC* show a certain regularity in the diminution of contamination with distance, whereas *EC*, *ED* and *WD* are rather irregular. Before drawing any conclusions from these results it is therefore necessary to submit them to an analysis of variance based on the angular transformation.

Table 8 shows the summarized results of this analysis. Beside the error variance calculated from the results is given the theoretical error variance of ϕ from the formula $820{\cdot}7/n$ where n is the harmonic mean of the sample sizes for each square. This theoretical error variance is generally much lower than that calculated from the observations, indicating that there is a real difference between the amounts of contamination occurring in the progeny of different plants at the same isolation distances in the same squares. This could arise through variation in degree of self-incompatibility, variation in the

distribution of the seed set with respect to time (this would only be effective in causing variation in contamination if contamination itself varied with time), or variation within the square of varietal mass which would be produced by variation in the floral mass of individual plants.

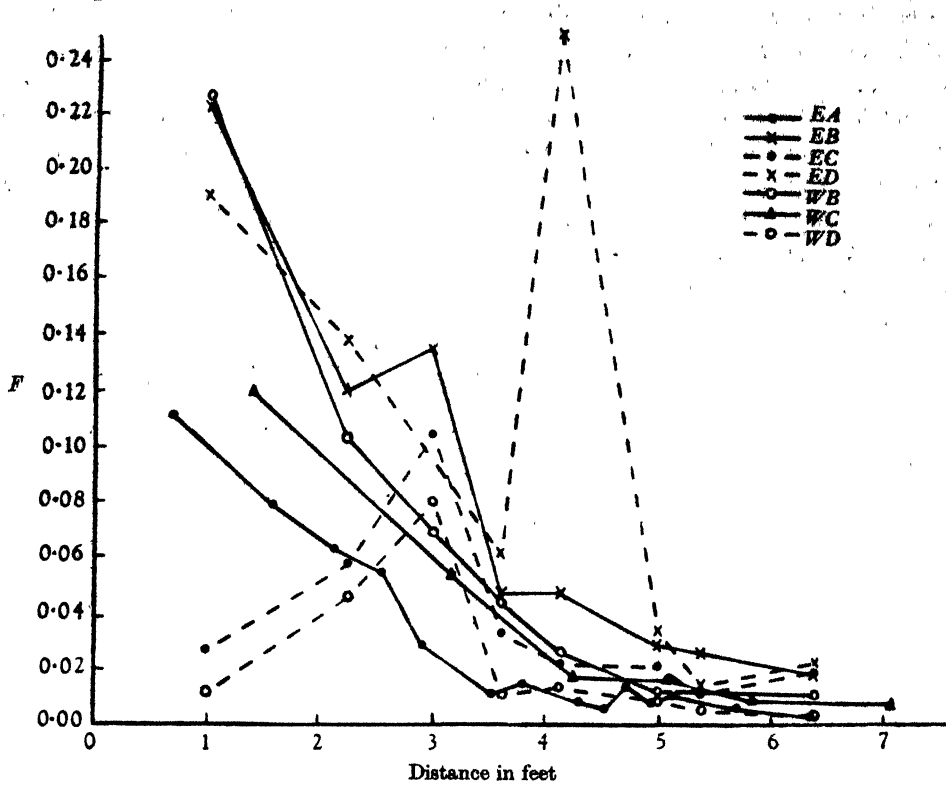

Fig. 7. (Exp. 4). Contamination F plotted against distance in ft. for seven squares. Entire lines represent squares with highly significant distance variances; broken lines, squares with less significant distance variances.

Table 8. *Summary of the analyses of variance of the radish density squares based on the angular transformation of* F

Square	Distance *MS*	Distance *N*	Error *MS*	Error *N*	Error $V\phi$	Variance ratio	Probability
EA	168·6699	13	26·7179	84	10·7952	6·31	Very small
EB	267·0905	7	23·0831	50	4·6966	11·57	Very small
EC	39·9888	7	18·0076	15	3·0333	2·22	0·05–0·2
ED	126·4594	6	33·6520	4	22·5545	3·76	0·05–0·2
WB	389·1958	7	39·2327	44	4·5990	9·92	Very small
WC	179·0683	5	10·8679	29	2·0514	16·48	Very small
WD	27·9322	7	1·4312	7	2·0858	19·52	<0·001

The column headed $V\phi$ is the error variance of the angular transformation due to sample size according to the formula $820{\cdot}7/n$ where n is the harmonic mean of the sample sizes of the squares.

The variance ratios of mean square for distance to mean square for error are very high in *EA*, *EB*, *WB* and *WC*. These are therefore reliable sources of data for studying the relation between distance and contamination. In *EC* and *ED* variance ratios are low. In *WD* though high it is based on an abnormally low error mean square, lower even than the theoretical variance calculated from the sample size. If we take the more normal error

mean square for *WC* which includes a sample size error variance of equal magnitude the new variance ratio has a probability of 0·05 which is very much less significant than that for *EA*, *EB*, *WB* and *WC*. We are therefore justified in only considering these last four squares for further analysis of the distance effect.

In spite of the great contrast in range of isolation distances (0·7–7 ft. in this experiment as against 20–580 ft. in the long-distance experiment) the essential shapes of the curves relating contamination and distance are the same.

One can also see at a glance that there is considerable variation between squares in the general level of contamination. A figure representing this can be obtained by summing the proportions of contamination of all plants in a square and dividing by the number of Icicle plants at the centre. This gives us the following set of figures:

	Density	*A*	*B*	*C*	*D*
	E	0·3692	1·0169	0·4394	1·0151
Replication	*W*	—	0·7855	0·6404	0·4384

It is seen that though the variation is considerable there is no obvious trend according to the replication or the density.

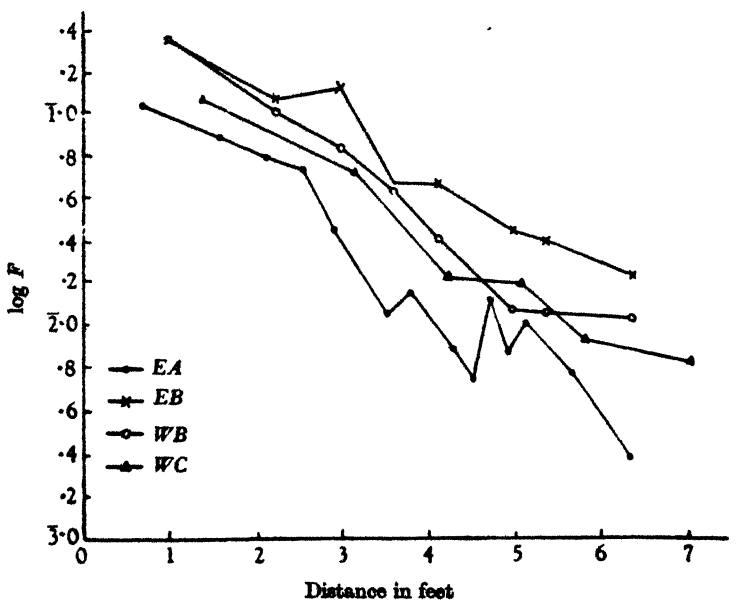

Fig. 8 (Exp. 4). Log *F* plotted against distance in ft. for the four most significant squares.

In examining the effects of density on contamination we must therefore eliminate variation in degree of contamination between squares. This can be done by taking logarithms of the contamination proportions. When plotted on a graph the relation between log contamination and distance will have a slope corresponding to the relative rate of decrease of contamination, i.e. independent of the absolute amounts.

Such curves are shown in Fig. 8. It is clear that all four curves are essentially parallel. This means that density has not been shown to have any effect on the spread of contamination, for an increase in the spread of contamination with decreasing density would cause the slope of *EA* to be greater than that of *EB* and the slope of *WB* greater than that of *WC*. This might at first appear to conflict with Butler's conclusions (1943) regarding the method of action of bees, which led one to expect sparse spacing of flowering plants to

cause the bees to forage more widely and produce more widespread contamination. But this would only be so if the number of bees per plot were constant, whereas they were in fact free to distribute themselves as they pleased. The above results suggest that under these conditions the bees tend to distribute themselves in a way (similar to the economic law of supply and demand) such that all foraging bees would have approximately equal returns of nectar or pollen for the same expenditure of energy. In this way the sparser planted squares would have a correspondingly sparser population of pollinators and the relative decrease of contamination with increase in distance would remain constant irrespective of density of planting.

These results were corroborated by an earlier experiment with turnip. Here the error variance was higher and the results therefore were less reliable, but there was again no evidence of any effect of density of planting on spread of contamination.

Summary

1. There are two types of contamination of seed crops, mechanical admixture of seed and cross-pollination. The latter is the more difficult to eradicate.

There are four main variables affecting cross-pollination between varieties: (i) the breeding system of the species; (ii) isolation distance; (iii) varietal mass; (iv) pollinating agent. Of these (iii) and (iv) have been most neglected.

There is little work available entailing a systematic study of these four factors. There is, therefore, a need for such a study of the independent effects of these factors and their interactions.

2. Using two insect-pollinated self-compatible species, radish and turnip, and suitable amounts of plants of the contaminating and contaminated varieties, the effect of distance on isolation is similar over a range of from 0 to 160 ft. At first there is a rapid reduction in contamination with increasing distance, but there is a progressive reduction in the decreases in contamination produced by repeated increases in isolation distance. In the experiment most conducive to contamination this fell from 60 % at 20 ft. to 13 % at 80 ft., but at 140 ft. it was still 6 %. In this same experiment, involving greater isolation distances than the others, on increasing the isolation distance from 160 to 580 ft. there was no evidence of a further decrease in contamination, which remained in the neighbourhood of 1 %. As there are *a priori* reasons for expecting some decrease it must be presumed to be too slight for detection.

3. The mass of a variety (here altered by varying the number of rows from 1 to 12), and the spatial arrangement of the plants, are as important in their effects on contamination as isolation distance. Mass is also similar to isolation distance in its mode of action, in that constant increases in the number of plants of a variety growing together produce progressively smaller decreases in its contamination.

4. When pollinating insects are free to distribute themselves over a crop they do so in such a way that density of planting has no effect on the rate of decrease of contamination with increasing isolation distance.

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REFERENCES

BALLS, W. L., TEMPLETON, J., BROWN, C. H. & KILAIN, M. (1929). The natural crossing of cotton flowers in Egypt. *Bull. 89 of the Egyptian Min. of Agric.*

BATEMAN, A. J. (1946). Genetical aspects of seed-growing. *Nature, Lond.*, **157**, 752.

BUTLER, C. G. (1943). Management of (bee) colonies for pollination. *Ann. Appl. Biol.* **30**, 195.

CRANE, M. B. & MATHER, K. (1943). The natural cross-pollination of crop plants with particular reference to the radish. *Ann. Appl. Biol.* **30**, 301.

CURRENCE, T. M. & JENKINS, J. M. (1942). Natural crossing in tomatoes in relation to distance and direction. *Proc. Am. Soc. Hort. Sci.* **41**, 273.

HASKELL, G. (1943). Spatial isolation of seed crops. *Nature, Lond.*, **151**, 307.

HAYES, H. K. & IMMER, F. R. (1942). Methods of plant breeding (for Regul. of Minnesota Crop Improv. Assn. on 273.)

SNEDECOR, G. W. (1946). *Statistical Methods* (4th ed.). Iowa State Coll. Press, Ames, Iowa.

THE THEORY OF A CLINE

BY J. B. S. HALDANE, F.R.S.

The intensity of natural selection, that is to say the relative fitness of several genotypes or phenotypes of the same species, can be estimated in several ways. Direct estimation is only possible in men, where, for example, 100 haemophilics or achondroplasics can be followed from birth, and their progeny compared with that of 100 normals. Similar observations in plants and animals are only possible under artificial conditions. Several indirect methods are possible. Some are dynamical. For example, the rate of spread of melanic mutants in industrial districts is roughly known, and a little is known as to the disappearance of mutants released in nature.

Others are statical. Thus on the basis of population counts Fisher (1939) showed that heterozygous dominants of *Paratettix* are fitter than the recessive type, homozygotes less so. This gives rise to balanced polymorphism. In some polymorphic species the frequency of the types is a function of the geographical situation of the populations studied. And it is sometimes found that the frequency of one type increases fairly steadily in a certain direction. Thus the Arctic skua, *Stercorarius parasiticus*, exists in two sharply distinguished types, a pale and a dark, probably genetically determined. The frequency of the pale type increases from about 25 to 75% as the North Pole is approached (Southern, 1943). Huxley (1939, 1942) has called a gradient of this kind a cline, and cites numerous examples. It will be shown that in certain cases a cline can give quantitative information regarding the intensity of natural selection.

A cline may be due to migration from the centre of origin of a gene. The cline in the frequency of the *B* agglutinogen in our own species with a maximum in central Asia appears to be of this type. Here there is no evidence that *B* makes for greater fitness in central Asia than in Europe or China, and the situation is probably unstable. This human cline may well disappear in a few thousand years as a result of interbreeding, and will almost certainly become less intense. On the other hand, the cline of increasing human skin colour towards the tropics may well have a selective value, and is therefore more likely to persist.

But a cline may also be due to the fact that one type is favoured by selection in one part of the habitat, the other in another part. Random migration serves to keep the population mixed, either throughout its area or in a border zone. A [illegible] case was analysed by Sumner (1929*a*, *b*, 1932) and Blair (1944). The deer-mouse, *Peromyscus polionotus*, inhabits Florida and Alabama. On the sandy beaches of the Gulf of Mexico it is represented by the lighter coloured subspecies *leucocephalus* and *albifrons*, which are less visible than the type against a background of white sand, and more so against ordinary soils. This is believed to give them an advantage in their special habitats, and a disadvantage elsewhere. The difference is partly, but not wholly, due to a dominant gene, **Wc**, causing white cheeks in the coastal subspecies. The subspecies intergrade with the type over a belt about 40 miles wide stretching inland from the beaches. There is reason to think that the situation is fairly stable, the gene **Wc** being advantageous on the beaches and harmful far inland, and similarly for the other genes present in the coastal

subspecies. Such a situation could be due to one-way migration from a crowded to a less crowded area. Since mice of the subspecies *polionotus polionotus* seem to be rare on the beaches, this would imply that the beaches are overcrowded, or at least that mice leave them and do not enter them. However, as there is no published evidence to this effect we shall assume that migration is at random, that is to say, that a mouse is as likely to move towards the beaches as inland, regardless of its colour.

We can analyse such a case mathematically if we make the following assumptions:

(1) A species lives in an area which is supposed to be plane and infinite. Actually it must be so large that regions exist where there is no appreciable polymorphism.

(2) The density is equal through this area.

(3) An autosomal dominant **A** and its allelomorph **a** cause polymorphism.

(4) The plane is sharply divided by a straight boundary into two halves. x is the distance of any point from the boundary. In the half plane where x is positive, **aa** zygotes have a fitness $1+K$ times that of **AA** and **Aa**. In the other half plane their fitness is $1-k$. K and k are small and positive.

(5) The animals have an annual generation and one only.

(6) They migrate at random. A group of mice born at distance x breed at distances $x+t$, where t is symmetrically distributed about zero with unit standard deviation. That is to say, we take as our unit of distance the root of the mean square of the distances travelled by an animal between birth and breeding in the direction normal to the boundary. The distribution need not be normal provided it is symmetrical and has finite moments.

(7) Selection occurs at the place of breeding, not of birth. This assumption simplifies the argument but does not affect the result appreciably.

(8) Mating is at random between the different types.

(9) The population is in equilibrium. The frequency of the gene **a** in adults at a distance x from the boundary is y, the frequency of the recessive phenotype **aa** being therefore $z=y^2$.

It is to be noted that many of these assumptions can be relaxed by making suitable allowances. Thus a barrier which is difficult to pass would be the equivalent of an increased distance.

When y or z are plotted against x we must get a curve of the type shown in Fig. 1, $y \to 0$ as $x \to -\infty$, and $y \to 1$ as $x \to \infty$. When $x=0$, i.e. on the boundary, y has a definite value b which is later determined. Also dy/dx is continuous at the boundary, since any discontinuity would be smoothed out by migration. But d^2y/dx^2 changes sign abruptly at the boundary, when $x=0$.

First let us calculate the effect of migration. Since migration does not depend on genotype, we can consider the genes, not the zygotes, as migrating. Let $f(t)$ be the frequency distribution function of t, symmetrical about zero. At the point $x+t$ the gene frequency is

$$Y=y+t\frac{dy}{dx}+\frac{t^2}{2!}\frac{d^2y}{dx^2}+\dots.$$

As a result of 1 year's migration the frequency at x changes from y to

$$\int_{-\infty}^{\infty} Yf(t)\,dt=y+\frac{1}{2}\frac{d^2y}{dx^2}\int_{-\infty}^{\infty} t^2 f(t)\,dt+\frac{1}{4!}\frac{d^4y}{dx^4}\int_{-\infty}^{\infty} t^4 f(t)\,dt+\dots$$

$$=y+\frac{\overline{t^2}}{2!}\frac{d^2y}{dx^2}+\frac{\overline{t^4}}{4!}\frac{d^4y}{dx^4}+\dots.$$

For a normal distribution this is

$$y+\frac{\sigma^2}{2!}\frac{d^2y}{dx^2}+\frac{3\sigma^4}{4!}\frac{d^4y}{dx^4}+\frac{15\sigma^6}{6!}\frac{d^6y}{dx^6}+\ldots.$$

Provided K and k are sufficiently small and the distribution is not too leptokurtic, we can neglect terms after the second, and we have already assumed $\sigma^2=1$, so we have the familiar diffusion expression

$$Y=y+\frac{1}{2}\frac{d^2y}{dx^2}.$$

As a result of selection the ratios of the genotypes are altered, when $x>0$, from

$$(1-y)^2\,\mathbf{AA} : 2y\,(1-y)\,\mathbf{Aa} : y^2\mathbf{aa}$$

to

$$(1-y)^2\,\mathbf{AA} : 2y\,(1-y)\,\mathbf{Aa} : (1+K)\,y^2\mathbf{aa}.$$

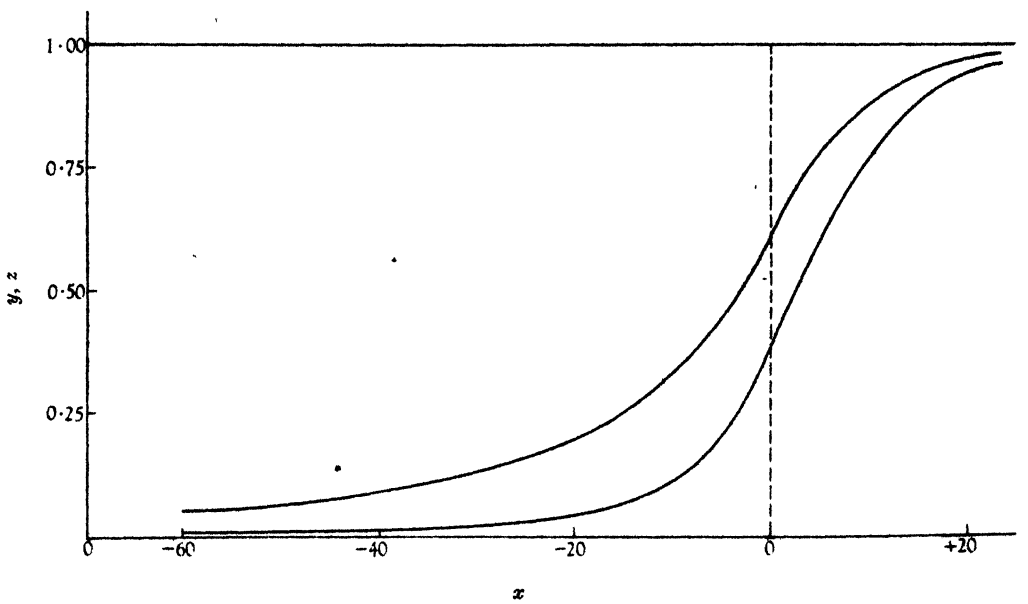


Fig. 1. Abscissa: distance from boundary, the unit being the root mean square of the distance migrated per generation. Ordinates: upper curve, frequency of recessive genes; lower curve, frequency of recessive zygotes. To the right of the boundary the fitness of **aa** is 1·01 that of **AA** or **Aa**, to the left it is 0·99 that of **AA** or **Aa**.

Thus the frequency of **a** is altered from y to $y+Ky^2/1+Ky^2$, or if K is small to $y+Ky^2\,(1-y)$ approximately. But selection and migration are in equilibrium. Hence

$$\left.\begin{aligned}\frac{d^2y}{dx^2}&=-2Ky^2\,(1-y)\quad(x>0),\\ \text{and similarly}\qquad \frac{d^2y}{dx^2}&=ky^2\,(1-y)\quad(x<0).\end{aligned}\right\}\qquad(1)$$

To solve this pair of differential equations, put $p=dy/dx$. Then, for $x>0$,

$$p\,\frac{dp}{dy}=-2Ky^2\,(1-y),$$

so
$$\tfrac{1}{2}p^2=\int p\,dp=-2K\int y^2(1-y)\,dy$$
$$=C-2K\left(\frac{y^3}{3}-\frac{y^4}{4}\right).$$

Since when $x\to\infty$, $y\to 1$ and $p\to 0$, $C=\frac{1}{6}K$, whence
$$\left(\frac{dy}{dx}\right)^2=\tfrac{1}{3}K\,(1-4y^3+3y^4)$$
$$=\tfrac{1}{3}K\,(1-y)^2\,(1+2y+3y^2).$$

Similarly, when $x<0$,
$$\left(\frac{dy}{dx}\right)^2=2C'+4k\left(\frac{y^3}{3}-\frac{y^4}{4}\right).$$

When $x\to-\infty$, $y\to 0$ and $p\to 0$, so $C'=0$, and
$$\left(\frac{dy}{dx}\right)^2=\tfrac{1}{3}ky^3\,(4-3y).$$

Now when $x=0$, $y=b$, and dy/dx has the same value for both branches of the curve. Hence
$$\tfrac{1}{3}K\,(1-4b^3+3b^4)=\tfrac{1}{3}k\,(4b^3-3b^4),$$
or
$$3b^4-4b^3+\frac{K}{K+k}=0. \tag{2}$$

This equation, giving the value of y on the boundary, has one and only one root between 0 and 1. It is readily solved by iterating
$$b=\left[\left(1+\frac{k}{K}\right)\left(\frac{4}{b}-3\right)\right]^{-\frac{1}{3}}.$$

Values of b in terms of K/k are given in Table 1.

Table 1

K/k	0	0·0037	0·0279	0·0913	0·2183	0·4545	0·9055	1	1·5753	2·821	4·531	8·259	18·49	∞
b	0	0·1	0·2	0·3	0·4	0·5	0·6	0·6143	0·7	0·75	0·8	0·85	0·9	1

It will be seen that even when selection is much more intense in one area than another, an appreciable number of genes will diffuse into the area of intense selection against them. The value of dy/dx at the boundary is
$$\left[\frac{Kk}{3\,(K+k)}\right]^{\frac{1}{2}}.$$

When $x>0$,
$$\frac{dy}{dx}=\sqrt{\frac{K}{3}}\,(1-y)\,\sqrt{(1+2y+3y^2)},$$

so
$$x=\left(\frac{3}{K}\right)^{\frac{1}{2}}\int_b^y\frac{du}{(1-u)\,\sqrt{(1+2u+3u^2)}},$$

or if
$$v=\frac{1}{1-u},$$
$$x=(2K)^{-\frac{1}{2}}\int_{1/(1-b)}^{1/(1-y)}\frac{dv}{\sqrt{[(v-\frac{2}{3})^2+\frac{1}{18}]}}$$
$$=(2K)^{-\frac{1}{2}}\Big[\sinh^{-1}(3\sqrt{2}v-2\sqrt{2})\Big]_{1/(1-b)}^{1/(1-y)}$$
$$=(2K)^{-\frac{1}{2}}\left[\sinh^{-1}\left\{\frac{\sqrt{2}\,(1+2y)}{1-y}\right\}-\sinh^{-1}\left\{\frac{\sqrt{2}\,(1+2b)}{1-b}\right\}\right].$$

When $x<0$,
$$\frac{dy}{dx}=\left(\frac{k}{3}\right)^{\frac{1}{2}} y^{\frac{1}{2}}(4-3y)^{\frac{1}{2}},$$
so
$$x=k^{-\frac{1}{2}}\int_y^b \frac{du}{\sqrt{[u^2(\frac{4}{3}-u)]}},$$
or if
$$v=\sqrt{\left(\frac{1}{u}-\frac{3}{4}\right)},$$
$$x=\sqrt{3}\int_{\sqrt{(1/y-\frac{3}{4})}}^{\sqrt{(1/b-\frac{3}{4})}} dv$$
$$=\frac{1}{2}\left[\sqrt{\left(\frac{3(4-3b)}{b}\right)}-\sqrt{\left(\frac{3(4-3y)}{y}\right)}\right].$$

We may now suppose that m^2 is the mean square of random migration distance, so that the root mean square in the x direction is $m/\sqrt{2}$. We then have

$$\left.\begin{aligned}
&\frac{2K^{\frac{1}{2}}}{m}x=\sinh^{-1}\left[\frac{\sqrt{2}(1+2y)}{1-y}\right]-\sinh^{-1}\left[\frac{\sqrt{2}(1+2b)}{1-b}\right] \quad \text{for } x>0,\\
\text{and}\quad &\frac{2}{m}\sqrt{\left(\frac{2k}{3}\right)}x=\sqrt{\left(\frac{4}{b}-3\right)}-\sqrt{\left(\frac{4}{y}-3\right)},\\
\text{or}\quad &y=\frac{bm^2}{m^2-mx\sqrt{[\frac{2}{3}k(4-3b)]}+\frac{2}{3}bkx^2}, \quad \text{for } x<0,
\end{aligned}\right\} \tag{3}$$

where b is given by equation (2), and $y=z^2$, z being the frequency of recessives.

Fig. 1 shows the frequencies y of the recessive gene, and z that of recessive zygotes, when $K/m^2=k/m^2=0{\cdot}01$. The interquartile range of y is about $0{\cdot}79m/\sqrt{K}$ when both quartiles are in the positive region, and $1{\cdot}27m/\sqrt{k}$ when both are in the negative region. Otherwise the value is intermediate. The interquartile range of $z=y^2$, i.e. the range of x where y varies between $\frac{1}{2}$ and $\sqrt{3}/2$, is $0{\cdot}811m/\sqrt{K}$, when both values of x are positive, $0{\cdot}590m/\sqrt{k}$ when both values are negative, and intermediate when one falls on each side of the boundary.

For *Peromyscus bairdii*, Blair's (1940) data suggest that m is about half a mile, though this may be incorrect by a factor of at least 2. I have taken the same value for *P. polionotus*, though this may well be incorrect. Sumner's data show that as one goes inland the different characters of *P. polionotus albifrons* disappear at different rates. The greater length of foot and tail, of which the former at least may be an adaptation to the softness of the sand, have disappeared at a station 20 miles inland from the coast (Crystal Lake), and the foot length is even rather below the continental values. On the other hand, there has been little change in the pigmentation, whichever of several measures of it is considered. This changes rather abruptly about 40 miles inland. The zone of maximum change corresponds to the boundary between 'deep, loose, salmon-coloured sand, very similar to beach or dune sand' on a Pliocene formation, and 'red or brown loams' derived from older limestones. The boundary passes through Round Lake. Several measures of pigmentation were made, notably the coloured area, the pigmentation of the basal zone of the ventral hairs, the length of the tail stripe, and the amount of red in the dorsal hair. The first of these probably gives the best indication of the gene **Wc**. It would seem that about 40% of the change in it occurs in about 10 miles between Round Lake and Chipley, in north-eastern Florida. We may take the interquartile range as being about 12 miles. If so, we have $m=\frac{1}{2}$, $d=12$, and since $d=0{\cdot}7m/\sqrt{k}$ or $0{\cdot}7m/\sqrt{K}$, approximately, we have

k or $K = m^2/2d^2 = 0{\cdot}001$ approximately. That is to say, a selective advantage of about 0·1 % on each side of the boundary would be sufficient to account for the observed cline. Since the mean coloured area on the boundary is 46 % of the distance from that of *albifrons* to that of *polionotus*, i.e. b equals about 0·73, it would seem that K and k are not very different in magnitude. Probably the selective advantage of **Wc** on the brilliant white sand of the beaches would be much greater.

Sumner (1929*b*) suggested that the beaches were not only the centre of origin of *P. polionotus albifrons*, but a centre of distribution from which it 'pressed inland, displacing and absorbing the darker form, *polionotus*, until its advance was halted by the centrifugal pressure of the latter'. If so we might expect that all the distinguishing characters of the subspecies would vary together with the distance from the coast. This is not the case. For example, the mean amount of red in the coat has reached 70 % of its inland value at Round Lake. If the characters are due to different genes subject to different intensities of selection, we should expect to find the situation actually observed. Sumner (1929*b*) pointed out that 'selective elimination, on the basis of concealing coloration, is far from intense among these animals'. He added: 'The discrimination on the part of their enemies would have to be well-nigh absolute in order to maintain a condition such as we actually find here.' If the calculation made above is even roughly correct this is not so. Even if K and k were of the order of 1 %, selection could only be detected with certainty by observations on tens of thousands of animals. It might well be found that a particular predator killed say 10 % more of light than dark mice on a dark ground, but it would then have to be shown that this predator accounted for 10 % of all deaths before an intensity of selection of 1 % was established, and the possibility of a counterbalancing advantage of light colour would have to be considered.

It must be remarked that other explanations of the observed facts are possible. Thus it may be that from time to time overcrowding on the beaches causes a wave of migration, and that the populations observed by Sumner are merely the remains of the last wave. If so the frequencies in a given place should vary greatly from year to year.

Theory of a cline in the absence of interbreeding

Consider two species which do not interbreed, but migrate at random at the same rate. Let y be the frequency of species A at distance x from the boundary, and let the relative fitness of A be $1+K$ in the positive area, $1-k$ in the negative area. Then in the positive area selection would increase y to $[(1+k)\,y]/[1+ky]$ or $y+ky\,(1-y)+O\,(k^2)$. Thus equations (1) are replaced by

$$\left.\begin{aligned} \frac{d^2y}{dx^2} &= -2Ky\,(1-y) \quad (x>0), \\ \frac{d^2y}{dx^2} &= 2ky\,(1-y) \quad (x<0). \end{aligned}\right\} \tag{4}$$

Hence

$$\left(\frac{dy}{dx}\right)^2 = \tfrac{2}{3}K\,(1-3y^2+2y^3) \quad (x>0),$$

$$\left(\frac{dy}{dx}\right)^2 = \tfrac{2}{3}k\,(3y^2-2y^3) \quad (x<0).$$

Hence the boundary value of x is given by

$$2b^3-3b^2+\frac{K}{K+k}=0, \tag{5}$$

and equations (3) are replaced by

$$\left.\begin{aligned}\frac{\sqrt{K}\,x}{m}&=\tanh^{-1}\sqrt{\left(\frac{1+2y}{3}\right)}-\tanh^{-1}\sqrt{\left(\frac{1+2b}{3}\right)} \quad (x>0),\\ \frac{\sqrt{k}\,x}{m}&=\tanh^{-1}\sqrt{\left(1-\frac{2b}{3}\right)}-\tanh^{-1}\sqrt{\left(1-\frac{2y}{3}\right)} \quad (x<0).\end{aligned}\right\} \tag{6}$$

Hence the interquartile ranges of y in the positive and negative regions are $0{\cdot}664m/\sqrt{K}$ and $0{\cdot}441m/\sqrt{k}$ respectively. It is noteworthy that these are only slightly less than the ranges found with interbreeding and dominance. In all four cases, if m is the root mean square range per generation, and d the interquartile distance, the coefficient of selection ranges between $0{\cdot}657m^2/d^2$ and $0{\cdot}195m^2/d^2$. This is a rather small range in practice.

DISCUSSION

The example given is at best approximate, and may be wholly fallacious. The method would, however, be reliable if adequate data were available, and it is hoped that the possibility of using them may stimulate their collection. An ideal set of data would include the following:

(1) Data on the frequency of different phenotypes over the area covered by the cline, especially near any conspicuous boundary.

(2) Data on the genetics of the character concerned. It is however to be noted that if a clear-cut difference is due to a single gene substitution, it makes very little difference whether this gene is dominant or recessive, since for a given intensity of selection the interquartile range is only 37 % longer in the region where recessives are favoured than in that where dominants are favoured.

(3) Evidence that mating is at random, or an estimate of the homogamy. This again is unimportant, since complete homogamy will only slightly increase the intensity of the cline, bringing it to the level characteristic for two different species.

(4) Data on migration, designed to give the mean square distance m^2 migrated per generation, and evidence that migration is random in direction, and independent of phenotype.

(5) Data over a number of years, to test whether the frequencies and migration rates are fairly stable.

The intensity of selection in the region including the quartiles, i.e. where the frequencies of one phenotype are 25 and 75 %, is then about $m^2/2d^2$, where d is the interquartile distance.

I have only made the calculation for a sharp boundary. In many cases there is no sharp boundary. Conditions change quite gradually, and the appropriate equation is something like

$$\frac{d^2y}{dx^2}=2kx\,y^2\,(1-y),$$

which does not appear to be simply soluble. However, a comparison of the interquartile and migration ranges should give the order of magnitude of the selection needed to keep

the cline in being, and this is all that can be expected until extensive data are available, particularly on the difficult subject of random migration, which may be expected to vary very greatly in different years.

It should nevertheless be possible, without very extensive work, to say whether a given cline suggests selective intensities of the order of 10 or 0·01 %.

It is perhaps worth commenting on the low values of K and k which are found. They imply that in a mixed population it would take about 16,000 generations to change the percentage of white-cheeked mice from 1 to 99 % or conversely. Hence it is probable that the light varieties were formed under the action of much more intense selection on the beaches.

Summary

Where one phenotype is favoured in one area and another phenotype in a neighbouring area, the character in question may be expected to show a cline in the neighbourhood of the boundary. On certain assumptions the relation between the intensity of selection, the mean distance migrated per generation, and the slope of the cline can be calculated. The relation is used for a provisional calculation of intensities of selection in a population of *Peromyscus polionotus*. These have the very low value of about 0·1 %.

REFERENCES

Blair, W. F. (1940). A study of prairie deermouse populations in southern Michigan. *Amer. Midl. Nat.* **24**, 273.

Blair, W. F. (1944). Inheritance of the white cheek character in mice of the genus *Peromyscus*. *Contr. Lab. Vert. Biol. Univ. Mich.* **25**.

Fisher, R. A. (1939). Selective forces in wild populations of *Paratettix texanus*. *Ann. Eugen., Lond.*, **9**, 109.

Huxley, J. S. (1939). Clines: an auxiliary method in taxonomy. *Bijdr. Diirk.* **27**, 491.

Huxley, J. S. (1942). *Evolution. The Modern Synthesis.* London.

Southern, H. N. (1943). *The Two Phases of* Stercorarius parasiticus *Linnaeus*, pp. 443–85.

Sumner, F. B. (1929*a*). The analysis of a concrete case of intergradation between two subspecies. *Proc. Nat. Acad. Sci., Wash.*, **15**, 110.

Sumner, F. B. (1929*b*). The analysis of a concrete case of intergradation between two subspecies. II. Additional data and interpretations. *Proc. Nat. Acad. Sci., Wash.*, **15**, 481.

Sumner, F. B. (1932). Genetic, distributional, and evolutionary studies of the subspecies of deermice (*Peromyscus*). *Bibl. Genet.* **9**, 1.

GENETICAL AND CYTOLOGICAL STUDIES OF *MUSA*

IX. THE ORIGIN OF AN EDIBLE DIPLOID AND THE SIGNIFICANCE OF INTERSPECIFIC HYBRIDIZATION IN THE BANANA COMPLEX

By K. S. DODDS and N. W. SIMMONDS

With an addendum on the Nomenclature of Edible Bananas

By E. E. CHEESMAN, *Department of Botany, Imperial College of Tropical Agriculture, Trinidad, B.W.I.*

(With Plate 11 and Ten Text-Figures)

1. Introduction

The cytology of five established edible diploid bananas was described by Dodds (1943). One of them appeared to be of hybrid origin and the suggestion was made that a similar plant might be synthesized by crossing *Musa Balbisiana* with an edible type of *M. acuminata*. This cross has now been made and a plant has been raised which closely resembles phenotypically the edible diploid in question. It is the purpose of the present paper to establish the resemblance and consider the significance of interspecific hybridization and polyploidy in the evolution of the banana complex.

The parents used were *M. Balbisiana* Colla: clone Ceylon (I.R. 100), and the edible diploid I.R. 143 (type 32 in the I.C.T.A. collection of edible types). The latter, apart from its edible fruits, very closely resembles certain strains of *M. acuminata* Colla, of which species it is to be regarded as a parthenocarpic form (Dodds, 1943; Dodds & Simmonds, 1948). It was received from Malaya where it is known as Pisang Lilan. The experimental hybrid, S.H. 62(1), is one member of a family of five plants, the cytology of which has previously been described (Dodds & Simmonds, 1946). The established edible diploid with which S.H. 62(1) is compared was obtained from the Trinidad Department of Agriculture in 1922. It was one of the survivors of a collection of edible bananas sent out from Kew to Dominica between 1898 and 1902 and passed on from Dominica to Trinidad under the label 'Guindy'. The clone has not, however, been identified with the well-known variety of that name in Madras (Watt, 1891, p. 293) and consequently is unnamed in the collection of *Musa* material at this Institution. It is designated type 20.

In the descriptions which follow, the various plant characters are treated in four groups, namely, vegetative parts, male buds, male flowers and fruits. Under each of these heads are given descriptions of I.R. 100 and I.R. 143, followed by notes on S.H. 62(1) with reference to its parents, and type 20 is commented upon with reference to the experimental hybrid. Thus some repetitive description is avoided and attention is focused on points of comparative interest.

2. The phenotypes

Vegetative parts

I.R. 100. Plant massive, free-suckering; bunches borne at 15–20 ft. (5–6 m.); leaves dark green above, slightly glaucous beneath; petioles enclosing the emerging leaf.

I.R. 143. Plant small, little-suckering; bunches borne at 5–8 ft. (1·5–2 m.); leaves light green above, glaucous below; petioles, sheaths and midrib flushed with red, glaucous; petioles open, not enclosing the emerging leaf.

S.H. 62(1). As I.R. 100 but with a slight trace of red in the pseudostem and petioles, especially in young suckers.

Type 20. Less vigorous than S.H. 62(1); otherwise similar but with a slightly greater tinge of red in the pseudostem and petioles.

Male buds

I.R. 100. *Male bud* (Text-fig. 2; Pl. 11, fig. 6), large, ovoid; *rachis* broad, with broad but low bract bases; *bracts* persistent, at length deciduous, broad, subacute, with a yellowish tip, crimson within, purple-glaucous without, tips strongly imbricate, never reflexed and rolled after lifting.

I.R. 143. *Male bud* (Text-fig. 1; Pl. 11, fig. 6), with broad shoulders, acute or acuminate; *rachis* narrow, with prominent, ridged bract bases; *bracts* deciduous, falling with or just after the flowers, small, relatively narrow, acute, lacking or with very slight differentiation at the tip, brownish crimson within, purple-glaucous without, reflexed and rolled after lifting, tips not at all imbricate.

S.H. 62(1). *Male bud* (Text-fig. 3; Pl. 11, fig. 7), large, intermediate in shape; *rachis* as in I.R. 143; *bracts* large, deciduous, intermediate in shape, lacking any differentiation at the tip, reflexed and rolled after lifting (Text-fig. 4), tips markedly imbricate.

Type 20. *Male bud* (Text-fig. 5; Pl. 11, fig. 7), similar to S.H. 62(1) but rather smaller and narrower and less 'shouldered'; *rachis* similar; *bracts* smaller, similar in shape but with a small yellowish tip, deciduous, not or at most very slightly reflexed and rolled after lifting, tips markedly imbricate.

Male flowers

I.R. 100. *Perianth* red-purple veined with orange-yellow teeth; *free tepal* large, with a very faint reddish flush, lacking point; *stamens* longer than the style and perianth; *style* robust, colourless with large stigma; *abortive ovary* slightly tapered with a red flush near base.

I.R. 143. *Perianth* white with yellow teeth; *free tepal* small, colourless with short point; *stamens* longer than the style and perianth; *style* slender, violet below, orange above, with small orange stigma; *abortive ovary* markedly tapered.

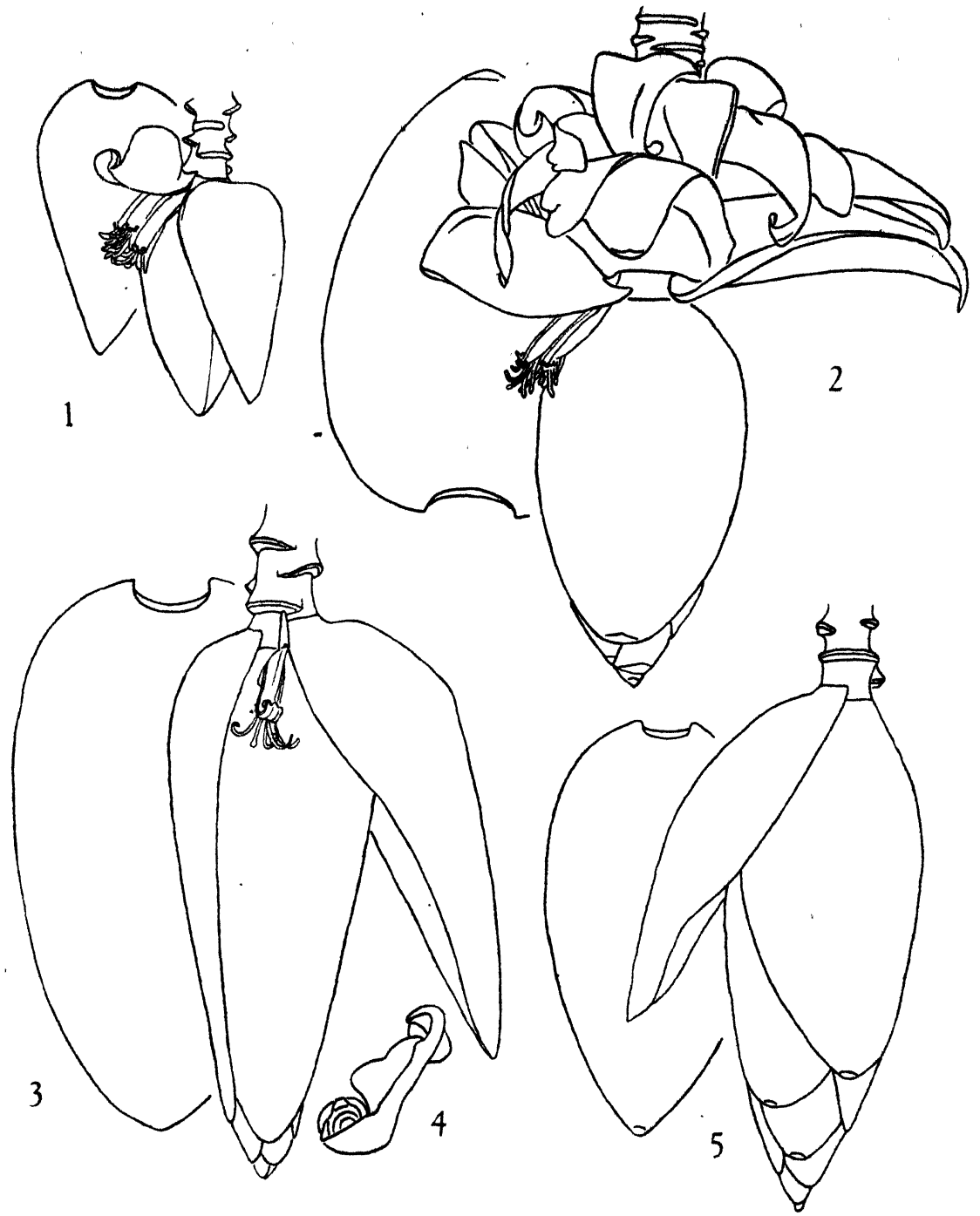
S.H. 62(1). *Perianth* red-purple veined with orange-yellow teeth; *free tepal* with red flush and well-developed yellowish orange point; *stamens* about same length as style, shorter than the perianth; *style* robust, pale violet below, orange above, stigma intermediate in size; *abortive ovary* intermediate in shape, flushed with red.

Type 20. *Perianth* paler in colour, tips yellow with no trace of orange; *free tepal* slightly more red; *stamens* longer than the style but little longer than the perianth; *style* similar though less robust; *abortive ovary* similar.

Floral dimensions are summarized in Table 1 and in Text-fig. 10.

Table 1. *Summary of lengths (mm.) of floral parts*

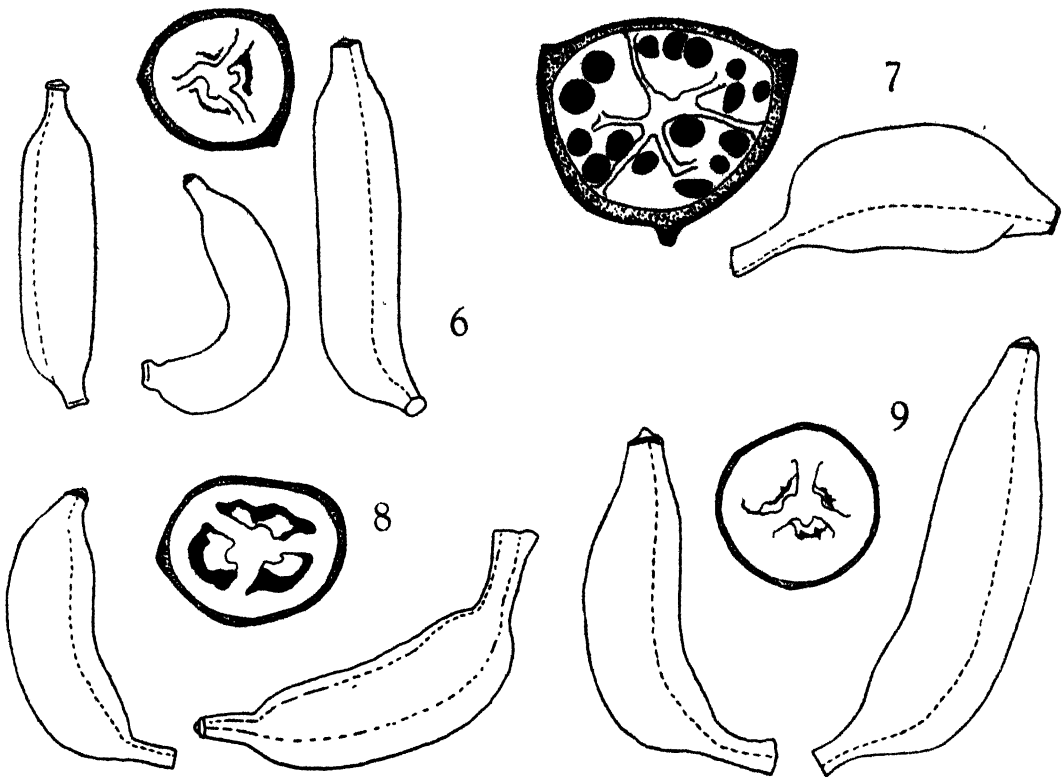
Organ	I.R. 100	I.R. 143	S.H. 62(1)	Type 20
Perianth	43	33	50	46
Stamens	50	38	47	48
Anthers	24	19	22	18
Style	40	33	46	43
Free tepal	23	15	22	22
Abortive ovary	16	9	20	15

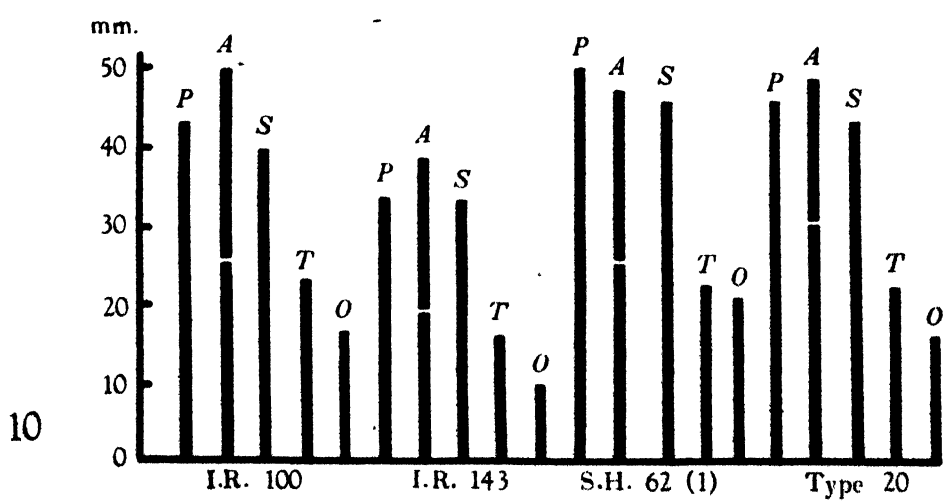


Text-figs. 1–5. Male buds and bracts (× 0·4). Text-fig. 1. I.R. 143. Text-fig. 2. I.R. 100. Text-fig. 3. S.H. 62(1). Text-fig. 4. Side view of re-rolled bract of S.H. 62(1) just before falling. Text-fig. 5. Type 20.

Bunch and fruit

I.R. 100. *Bunch* large, vertically pendulous, 5–9 hands, up to 18 or 20 fingers per hand, maturing in 4 months or more; *ovaries* glaucous, trilocular, with 330–60 ovules, four-rowed in each loculus; *secondary septa* appear early in fruit development; *mature fruit*





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Text-figs. 6–10. Fruits and floral dimensions (fruits ×0·4, sections of fruits ×0·8). Text-fig. 6. I.R. 143. Text-fig. 7. I.R. 100. Text-fig. 8. S.H. 62(1). Text-fig. 9. Type 20. Text-fig. 10. Graphical summary of floral dimensions—lengths (in mm.) of perianth, *P*; stamens, *A* (anthers and filament are distinguished by a break in the line); style, *S*; free tepal, *T*; and abortive ovary, *O*.

fully seeded, glaucous-green, flushed red at the base, short, broad, angled and pedicellate (Text-fig. 7; Pl. 11, fig. 1).

I.R. 143. *Bunch* small, horizontal, 2–5 hands, up to 10 or 12 fingers per hand, maturing in about 3 months; *ovaries* not glaucous, trilocular, with about 120–40 ovules, two-rowed in each loculus; *secondary septa* absent; *fruit* fully parthenocarpic, relatively long and narrow, hardly angled, subsessile (Text-fig. 6; Pl. 11, fig. 2).

S.H. 62(1). *Bunch* large, nearly vertically pendulous, 5–9 hands, up to 16 or 18 fingers per hand, maturing in 3–4 months; *ovaries* slightly glaucous, trilocular, with 200–50 ovules, irregularly four-rowed in each loculus; *secondary septa* absent; fruit fully parthenocarpic but with a relatively large amount of gelatinous material in the loculus, intermediate in shape, hardly angled, pedicellate, with red flush near base (Text-fig. 8; Pl. 11, figs. 3, 4).

Type 20. *Bunch* similar, maturing in 3–4 months; *ovaries* trilocular, with about 280 ovules, irregularly four-rowed in each loculus; *fruit* similar but slightly glaucous, with a very thin skin and of superior flavour (Text-fig. 9; Pl. 11, fig. 5).

3. S.H. 62(1) IN RELATION TO THE ORIGIN OF TYPE 20

From the preceding descriptions it will be seen that S.H. 62(1) is similar to I.R. 100 in vegetative vigour and in the size of its various parts. Buds, bracts and fruit are intermediate between those of the parents in shape. Several characters show dominance; for example, the hybrid is parthenocarpic and has the prominent bract bases and the habit of re-rolling and dropping the bracts found in the male parent. It has the heavy bunch and pedicellate fruits of I.R. 100 and shows, although rather irregularly, its four-rowed ovular arrangement. Ovular number and arrangement seem to be an important difference between *M. acuminata* and *M. Balbisiana*, the four-rowed arrangement being associated with the production of secondary septa early in fruit development.

With regard to male flowers, one 'new' character seems to have appeared in the hybrid, namely the long perianth of which the length is greater even than that in I.R. 100. The proportions of the various parts of the flower are quite altered for the perianth is longer than the style and stamens, of which the lengths are closer than in either parent (Table 1, Text-fig. 10).

Fresh flowers of I.R. 143, S.H. 62(1) and type 20 preserved in alcohol rapidly became discoloured and after a few hours had become dark brown. By contrast, flowers of I.R. 100 showed no such behaviour, being only slightly discoloured after many weeks. Whatever the nature of the reaction, it is clear the discoloration is dominant in the hybrid and that type 20 resembles it in this respect.

The comparison between S.H. 62(1) and type 20 shows that they differ in no important respect. The latter is rather less vigorous, the male bud is slightly less 'shouldered', and the imbrication of its bracts somewhat greater; the bunches of the two types are very similar, but the fruits of type 20 have thinner skins and a slightly superior flavour. There are also differences in various minor flower characters as previously described. However, the natural variability of both the parental species is quite sufficient to account for all these differences.

Before concluding that the production of S.H. 62(1) satisfactorily represents a synthesis of type 20, some consideration must be given to their cytological behaviour. Dodds (1943) has shown that metaphases of type 20 have from 3 to 10 bivalents with a mean of 6·7, while Dodds & Simmonds (1946) found an average of 10·0 bivalents per nucleus in

S.H. 62(1). The experimental hybrid was shown to be heterozygous for an interchange which gave occasional multiple associations at metaphase; and it may confidently be stated that were it not for this, the frequency of bivalents at metaphase would have been higher still—that is, somewhat over 10 bivalents per nucleus. This inference is supported by the fact that a closely comparable hybrid between *M. Balbisiana* and *M. acuminata* (S.H. 6; I.R. 100 × I.R. 53; Dodds & Simmonds, 1946) was not apparently heterozygous for interchange and had a mean of 10·2 bivalents per nucleus. Thus type 20 may be regarded as having about 3·5 bivalents per nucleus fewer than S.H. 62(1), when due allowance is made for interchange hybridity. This difference is thought to have resulted from the accumulation of structural hybridity in the chromosomes of type 20 during the course of its clonal existence (cf. Dodds & Simmonds, 1948). It seems likely that such a reduction in frequency of bivalents would readily occur in an interspecific hybrid in which initial differences between the component genomes were relatively great. Metaphase association may be supposed to result from chiasmata approaching in number the necessary minimum and, in consequence, it would be particularly susceptible to any additional structural hybridity which might accumulate.

Thus, though the cytological data provide no direct support for the hypothesis that the origin of type 20 is similar to that of S.H. 62(1), they are not incompatible with it. The conclusion seems justifiable that type 20 is the parthenocarpic product of interspecific hybridization between *M. Balbisiana* and *M. acuminata*. However, the species from which parthenocarpy derived cannot be named; all other edible diploids in the collection at this Institution are referred to *M. acuminata*, but more extensive collection might reveal the existence of edible diploid strains of *M. Balbisiana*.

Four siblings of S.H. 62(1) are similar to it phenotypically except in certain fruit characters; plant (2) is parthenocarpic with late development of edible pulp, while plants (3), (4) and (5) are non-parthenocarpic ('persistent'; Dodds & Simmonds, 1946, 1948). This segregation is characteristic of F_1 hybrid families of I.R. 143 and has been shown to depend upon the segregation of a dominant gene for parthenocarpy for which I.R. 143 is heterozygous (*Pp*) (Dodds & Simmonds, 1948). Furthermore, all four siblings showed varying degrees of restitution, but this was not detected in the male meiosis of S.H. 62(1) (Dodds & Simmonds, 1946).

4. Interspecific hybridization and the origin of edible triploids

Cultivated bananas (including 'plantains', see Addendum) from the Indo-Malayan region are, as a group, variable; but the phenotypic characters of none known to the authors suggest an origin from species other than *M. Balbisiana* and *M. acuminata*. The majority are triploid clones that may be assumed to have been vegetatively propagated since their origin, and hence to have preserved intact their original phenotypic characters. Somatic mutations are known (Cheesman, 1933; Larter, 1938; Cheesman & Dodds, 1942) but they affect only colour, habit, etc. and there is no reason to suppose that they seriously influence the floral characters diagnostic of the ancestral species. In short, obligatory vegetative propagation has ensured the preservation of relatively unchanged phenotypes.

It becomes necessary, therefore, to examine and, if possible, explain, first, the bispecific ancestry of the group and, secondly, the frequent origin of triploidy.

M. Balbisiana appears to be the most widely distributed species of *Musa*, occurring from Ceylon, through India and Burma, and southwards through the Malay Archipelago

as far as New Guinea. *M. acuminata* has been recorded from Burma, Thailand, Indo-China, the Malay Peninsula, Java and other islands of the East Indies, and, if further work confirms the view of Prof. E. E. Cheesman (unpublished) that *M. Banksii* F.v.M. is conspecific, its range extends to Queensland. These two species are therefore sympatric (Mayr, 1942) over a part of their respective ranges and natural crossing between them is a potentiality. The 'synthesis' of type 20 is evidence that such crossing has been an actuality.

Now while the phenotypes of certain triploids in the collection of *Musa* material at this Institution suggests that two species (*M. Balbisiana* and *M. acuminata*) are concerned in their ancestry, e.g. Mysore (type 6, I.R. 52), Silk Fig (type 17, I.R. 66), Pome (type 15, I.R. 54), etc., the phenotypes of others suggest in their cases only a single species. For example, Bluggoe (type 11, I.R. 12), Awak Legor (type 12, I.R. 90), King (type 13, I.R. 43) and Celat (type 14, I.R. 21) approximate to *M. Balbisiana* while Gros Michel (type 1, I.R. 31) and Rajah (type 18, I.R. 63) appear to derive from *M. acuminata*. Triploidy, therefore, has had various as well as frequent origins.

Dodds & Simmonds (1946) have described how, in certain interspecific crosses, diploid and tetraploid spores were formed as a result of a peculiar form of restitution arising from the partial or complete suppression of meiosis. The relative frequencies of production of haploid, diploid and tetraploid spores varied greatly between different crosses and between siblings within the same cross. All these hybrids were male sterile, but some yielded an abundance of polyploid seedlings ($3x$ and $5x$) on backcrossing with haploid pollen, thus showing that the polyploid spores were functional in the female. Moreover, it has recently been shown that the ratio of triploid to pentaploid seedlings varies between bunches of the same plant (S.H. 51(1)) pollinated at different times (Simmonds, 1948). Hence there is a variable incidence of meiotic breakdown, associated with hybridity, and dependent in degree and amount upon specific genotypic as well as environmental conditions (cf. Dodds & Simmonds, 1948).

The breakdown is characteristic of interspecific hybrids, but evidence accumulates to show that it occurs as a rare abnormality in almost any material (cf. Dodds & Pittendrigh, 1946, Table 7). And Dodds & Simmonds (1948) have described a single occurrence of this misbehaviour in the anthers of I.R. 143, a plant which may be regarded as a parthenocarpic strain of *M. acuminata* and which, as female parent, has given only two seedlings, both triploid (Dodds, 1943). It seems a reasonable inference, therefore, that the triploidy so prevalent among cultivated bananas is a result of the operation of this mechanism.

Of the hybrids between *M. Balbisiana* and *M. acuminata* raised at this Institution, S.H. 15 (I.R. 124 × I.R. 100) and S.H. 62 have proved sterile to all experimental pollinations (Dodds & Pittendrigh, 1946; Dodds & Simmonds, 1946). Recently, however, two seedlings have been raised from seeds from chance out-pollination of S.H. 62(2); one was diploid but the other died before its chromosome number was determined. S.H. 21 (I.R. 132 × I.R. 100) has produced only triploids (three) and S.H. 6 (I.R. 100 × I.R. 53) and S.H. 17 (I.R. 123 × I.R. 100) have produced only pentaploids (Dodds & Pittendrigh, 1946; Table 7). It is demonstrated, therefore, that the F_1 of this cross produces a range of diploid and polyploid offspring according to genetic (and environmental) conditions.

Hence the occurrence of polyploids of the *acuminata-Balbisiana* parentage is readily understood. That only triploids are found in nature, and not pentaploids, is to be related to the observation that there is a vigour maximum at triploidy (-tetraploidy) in *Musa*

(Simmonds, 1948). Pentaploid seedlings must occur, but the categorical statement may be made that none would have sufficient vigour to survive. And these considerations must apply to all polyploids whether of inter- or intra-specific origins.

A mechanism for the production of tetraploids is also known. Under experimental conditions, intensive pollination of triploids with haploid pollen yields—when any seed is produced—a majority of tetraploids, a few triploids and a small minority of heptaploids and (approximate) diploids (Cheesman & Dodds, 1942). The tetraploids are hardly, if at all, less vigorous than triploids. Nevertheless, no established tetraploids are known as yet, though about 40 triploids from the Indo-Malayan region have been examined. Furthermore, chromosome counts have been made of 12 edible types from certain Pacific Islands; these constitute taxonomically a rather distinct group from the Indo-Malayan edible types, but 11 were triploid ($3x=33$) and one was diploid ($2x=22$) (unpublished). Thus it can be said that tetraploids are rare, if not non-existent. An explanation of this is needed, for tetraploids are, at least theoretically, a potential source of triploids by backcrossing with haploid pollen.

5. Summary

1. Phenotypes of the following diploids ($2x=22$) are described: I.R. 100 (*Musa Balbisiana*: clone Ceylon), I.R. 143 (a parthenocarpic form of *M. acuminata*), S.H. 62(1) (a parthenocarpic F_1 hybrid between I.R. 100 and I.R. 143), and type 20 (an established edible diploid); the last two named are phenotypically very similar.

2. Phenotypic characters are thought to give reliable indications of origins, whence type 20 is inferred to have arisen from hybridization between *M. acuminata* and *M. Bal-*

3. The significance of interspecific hybridity, meiotic breakdown and polyploidy in the banana complex is discussed in relation to the evolution of established edible triploids.

REFERENCES

Cheesman, E. E. (1933). Mutant types of the dwarf banana. *Trop. Agriculture, Trin.*, **10**, 4–5.

Cheesman, E. E. & Dodds, K. S. (1942). Genetical and cytological studies of *Musa*. IV. Certain triploid clones. *J. Genet.* **43**, 337–57.

Dodds, K. S. (1943). Genetical and cytological studies of *Musa*. V. Certain edible diploids. *J. Genet.* **45**, 113–38.

Dodds, K. S. & Pittendrigh, C. S. (1946). Genetical and cytological studies of *Musa*. VII. Certain aspects of polyploidy. *J. Genet.* **47**, 162–77.

Dodds, K. S. & Simmonds, N. W. (1946). Genetical and cytological studies of *Musa*. VIII. The formation of polyploid spores. *J. Genet.* **47**, 223–41.

Dodds, K. S. & Simmonds, N. W. (1948). Sterility and parthenocarpy in certain diploid hybrids of *Musa* (in the press).

Larter, L. N. H. (1938). Banana varieties in Jamaica. *J. Jamaica Agric. Soc.* **42**, 460–8.

Mayr, E. (1942). *Systematics and the Origin of Species from the Viewpoint of a Zoologist.* London: Oxford Univ. Press.

Simmonds, N. W. (1948). Genetical and cytological studies of *Musa*. X. Stomatal size and plant vigour in relation to polyploidy. *J. Genet.* **49** (in the press).

Watt, G. (1891). *Dictionary of the Economic Plants of India*, **5**. Calcutta.

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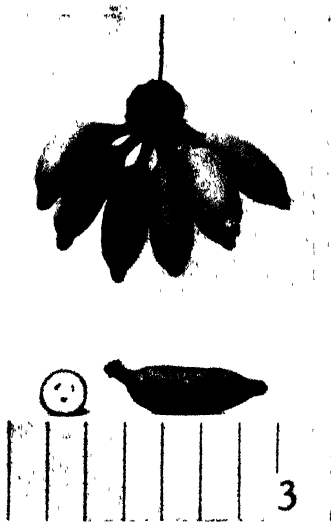
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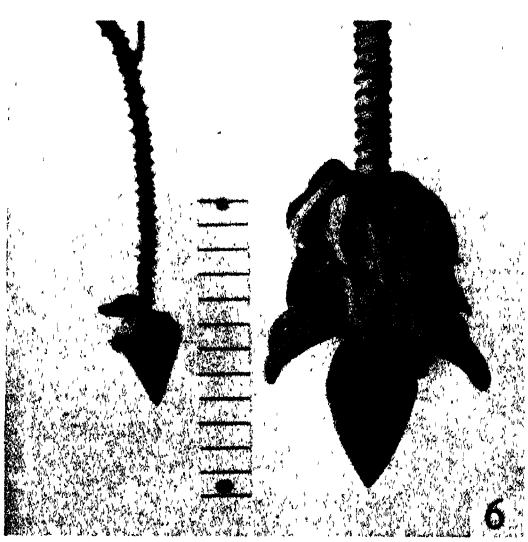
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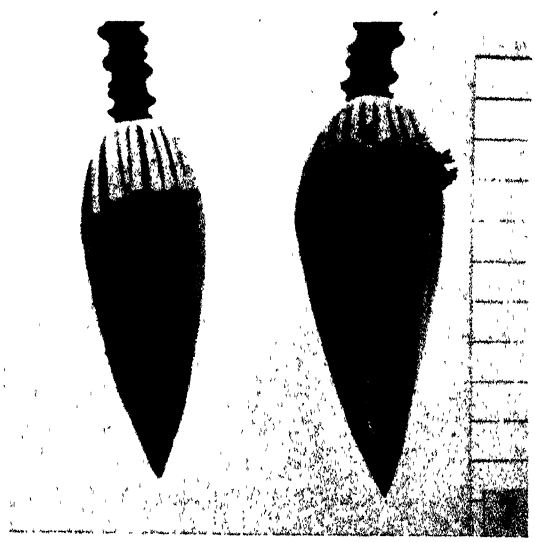

EXPLANATION OF PLATE 11

PLATE 11

Fruits and male buds; the scales are divided in inches.

Fig. 1. I.R. 100.
Fig. 2. I.R. 143.
Fig. 3. S.H. 62(1).
Figs. 4, 5. Type 20.
Fig. 6. Male buds of I.R 143 (left) and I.R. 100 (right)
Fig. 7. Male buds of type 20 (left) and S.H. 62(1) (right).

ADDENDUM

ON THE NOMENCLATURE OF EDIBLE BANANAS

BY E. E. CHEESMAN

Jour. of Genetics 48. 293

(1948)

This paper by Dodds & Simmonds has a special interest as a step toward the revision of the taxonomy and nomenclature of the edible bananas.

As the authors have implied, all the edible bananas from the Indo-Malayan region in the Imperial College *Musa* collection can be sorted by phenotype into three groups: (1) those that appear to derive from *M. acuminata* Colla, (2) those that appear to derive from *M. Balbisiana* Colla, (3) those that appear to have both species in their ancestry. We have recognized this division for some time, but before advancing it as a basis for classification wished for proof, first, that an edible banana variety could be bred from a cross between the two species mentioned, and secondly, that the variety would resemble some member or members of the third group sufficiently closely to support the hypothesis of their ancestry. With these proofs now in hand, it is time to discuss the correspondence between our three groups and the 'species' of current nomenclature.

Linnaeus, as is well known, used two specific names for edible bananas, *paradisiaca* and *sapientum*, and the subsequent application of the Linnaean epithets has varied widely with different authorities on *Musa*. In the most general usage at present, three 'species' are recognized: *M. paradisiaca* standing for 'plantains' (commonly understood as certain bananas of a starchy consistency, not palatable unless cooked), *M. sapientum* for all 'dessert bananas' except one variety distinguished by dwarf habit, and *M. Cavendishii* Lamb. for the said dwarf variety, otherwise known as the Canary or Chinese banana.

M. Cavendishii Lamb. can be very shortly dismissed. Dwarfness of habit by itself is clearly no ground for the recognition of a species, and it was shown several years ago (Cheesman, Wardlaw & Spencer, 1933) that the Cavendish banana can be connected, through a series of its own mutant forms, with a tall variety only distinguishable with difficulty from the well-known Gros Michel. Thus this name, though widely used in the literature, has no botanical justification and must be rejected in favour of one of the Linnaean names.

The distinction between plantains and dessert bananas as 'species' is likewise unsound botanically. The fruit quality depends on differences in the proportions of starch and sugars in the ripe fruit, and has undoubtedly a genetical basis, but differences of this kind are frequently varietal rather than specific, and in *Musa* certainly do not run parallel with specific characters. We have, therefore, to inquire into the original significance of the two Linnaean names.

M. paradisiaca L. is the older, and dates back to the first edition of *Species Plantarum* (1753) where the type was described simply as '*Musa* with a nodding spadix'. In *Systema Naturae*, 10th ed. (1759), where Linnaeus wished to distinguish a second kind of banana

which had come to his notice, and which also had a nodding spadix, he noted persistence of the male flowers, thus:

Musa with male flowers persistent. *paradisiaca*
Musa with male flowers deciduous. *sapientum*

This by itself is no better as a specific distinction in *Musa* than the starchiness of the fruit, but the mention of the persistent male flowers (an uncommon character) coupled with a reference in *Species Plantarum* to *Musa Cliffortiana* (a particular plant described from living material by Linnaeus in 1736) enables us to fix the type of *M. paradisiaca* with some confidence. The name was originally applied to a variety of cooking banana with persistent bracts, which is perhaps the commonest type of 'plantain', and the variety to which the name *M. paradisiaca* is still most commonly applied to-day.

So far, everything is straightforward. *M. paradisiaca* is the oldest name in the genus and unquestionably valid when applied to the plantain with persistent bracts and male flowers. But there are other plantains which do not have persistent bracts and male flowers, yet do not differ from *M. paradisiaca* in any more important character; the name must be extended to cover those. Then we find dessert bananas which, on the sum of their characters, must be regarded as belonging to the same species, and so the name must be extended again to cover some varieties of dessert bananas.

Now, the bananas (and plantains) covered by this extended use of the name *M. paradisiaca* L. are those which, in our opinion, are derived directly from the wild species *M. acuminata* Colla. They form the first of the classes enumerated above. According to a very strict interpretation of the rules of nomenclature, the epithet *acuminata* should not be used at all, but the older epithet *paradisiaca* should be employed for the wild as well as the cultivated forms. But there is precedent in treatments of some other genera containing crop plants for the use of one name for a group of cultivated varieties (cultigen) and another for the wild species from which they are *supposed* to be derived. The practice is very convenient, and it helps very much to reduce confusion, since the connexion is only a *supposition*, which, in *Musa*, in the presence of parthenocarpy, polyploidy, sterility and accumulated mutations in the cultigen is going to be exceedingly difficult, if not impossible, to *prove*.

It is submitted, therefore, that primarily as a matter of convenience, but also as a precaution against implications not entirely justified by the state of knowledge, the name *M. paradisiaca* L. should be restricted for the present to *cultivated* bananas (including plantains) considered conspecific with the Linnaean type. It will then include many, but not all, of the varieties commonly referred at present to *M. sapientum* L., and botanically it will indicate a cultigen *believed* to be derived from *M. acuminata*.

The type of *M. sapientum* L. is much more difficult to fix. In *Systema Naturae* (1759) where the name first appears, Linnaeus cites 'Ehr. sel. t. 21, 22, 23'. This is a reference to a volume of drawings painted by Ehret and published by Trew in 1750. The three plates represent one plant, and show respectively the habit, the female flowers and the ripe fruit. They are not easy to interpret, and do not strongly suggest any banana variety known to us; they show some, but not all, of the characters of *M. Balbisiana*. There is a clue to the identity of the depicted plant in a reference to Sloane's *Catalogus plantarum quae in Insula Jamaica sponte proveniunt* of 1696. It was evidently a banana variety which was in Jamaica at that date, and the number of varieties in the New World before 1700 was not very large. Among what seem to be the older introductions is type 17 of the I.C.T.A. collection, known in Trinidad as silk fig, which has been compared with a variety from

Madras called Rastali and judged identical. This is known in some countries as the apple banana; it is widespread in the New World to-day, and it comes nearer to Ehret's tab. 22 than any other West Indian variety. The identification is not entirely satisfactory, because tab. 23 shows fruits about 4 in. long by 3 in. in diameter, several of which are split. The fruit of the silk fig is not nearly so thick in proportion to its length; but for that matter, no West Indian banana at the present time has a fruit of the shape indicated, and unless the variety has been lost we must conclude that Ehret's fruit drawing is bad.

One thing certain about the plant depicted by Ehret is that it was not a form of *M. paradisiaca* as we have defined that cultigen. The name *M. sapientum* therefore cannot properly be regarded as a synonym of *M. paradisiaca* L., nor can it properly be used as the name of a subspecies. The combination *M. paradisiaca* subsp. *sapientum* used by K. Schumann and some other authorities is taxonomically unsound. *M. sapientum* subsp. *paradisiaca* used by J. G. Baker and others is even worse, violating a rule of nomenclature as well as the findings of taxonomy.

Another thing to be accepted about the original *M. sapientum* is that it had some of the characters of *M. Balbisiana* but lacked some others which we regard as diagnostic of that species. We should, therefore, almost certainly classify it in our third group of phenotypes if we had it in our collection to-day, and it would there be near to type 17 even if it proved distinct from that clone. Balancing all the probabilities, it seems more likely that Ehret drew his fruit a little out of proportion than that he selected an uncommon variety which has since disappeared, and identification of *M. sapientum* L. with our type 17, the apple banana or silk fig seems to be the most reasonable conclusion to be reached.

Now, type 17 is very close in phenotype to type 20, the differences being mainly such as we should expect from the greater vigour of the former. Evidence about the origin of type 20 may therefore be taken as relevant to the origin of type 17 likewise. The significance, in this connexion, of the facts established by Dodds & Simmonds lies in the strong support they afford to the view that the original *M. sapientum* of Linnaeus was an interspecific hybrid.

We are now confronted with a nice problem in the wider application of the name. Strictly, there are objections to its application to anything except the one banana of doubtful identity depicted by Ehret, and those who prefer to use Latin binomials only when a tolerably precise meaning can be attached to them will dislike the combination altogether. On the other hand, if we reject it entirely we have two classes of banana varieties for which no established 'cultigen' names are available, these being the class supposedly derived directly from *M. Balbisiana* and that believed to have originated in a cross, or crosses, between that species and *M. acuminata*. The two classes have certain characters in common which serve to mark them off quite definitely from *M. paradisiaca*, and it seems desirable that they should be distinguished from that fairly well-marked cultigen, and from each other. Yet to introduce two entirely new names would probably cause more confusion than it would clear, apart from being open to other objections.

As it seems impossible to combine accuracy, convenience and logical arrangement in the solution of this problem, and the first two considerations are the more important, I suggest that our three groups of banana varieties may be designated as follows:

Group	Putative origin	Cultigen name
1	*M. acuminata* Colla	*M. paradisiaca* L.
2	*M. Balbisiana* Colla	*M. Balbisiana* (*sensu lato*)
3	Hybrids of *M. acuminata* × *Balbisiana*	*M. sapientum* L. (*sensu lato*)

The illogical feature of this arrangement is the extension of *M. Balbisiana* to cover edible forms, coupled with the refusal to extend *M. paradisiaca* to cover wild and fully seeded forms. But it may serve its purpose until further research suggests a better.

A note may be added on the confusion between *M. Balbisiana* and *M. sapientum* in the older literature. The species which we call *M. Balbisiana* has been recognized by several authorities as a parent of some cultivated bananas, but failure on their part to realize the bispecific ancestry of the group led them to transfer back the name *sapientum* from the cultivated to the wild form without enquiring further into the relationship. Roxburgh, in particular, dealing with the forms of *Musa* in India in his *Flora Indica* (1824) gave an excellent description of *M. Balbisiana* but called it '*Musa sapientum* (the wild sort)', and concluded that this was 'the original wild *Musa* from which...all the cultivated varieties of both plantain and banana proceed'.

Sulpiz Kurz (1865) came nearer to the truth with these words: 'The continent of India is usually designated as the native country of the cultivated kinds of plantains, a view that I can entertain only partially. After long enquiries into this question on the Malayan islands I have come to the conclusion that the cultivated Plantains belong to several botanically different species and also, that the original species, from which most of the numerous varieties now in cultivation in the Archipelago descend, is a Malayan species.... *Musa sapientum*—a second kind of plantain remarkable for its numerous varieties, is a true continental species, occurring in the forests from Behar up to the Himalaya.'

If we read '*M. Balbisiana*' for '*M. sapientum*' in this quotation, it appears that our present views on banana classification are very similar to those held by Kurz. The ranges, both of his Malayan species (*M. acuminata*) and of his '*M. sapientum*', have been found to be wider than he knew, and the possibility of their crossing is perhaps new since his day; but he noticed the essential fact of bispecific origins which many missed. It is a pleasure to pay tribute to Kurz, whose field observations were strangely underestimated or neglected by later systematists working on *Musa*, but have been highly valuable in our more recent studies.

The wild species that he and Roxburgh had in mind, however, does not agree sufficiently with the type of *M. sapientum* to bear the same name. This wild species, although described and figured by Rumphius in the *Herbarium Amboinense* (1750), was not given a name valid by modern rules until Colla (1820) named it *M. Balbisiana*. Colla's name has likewise been neglected by later systematists, although it applies to the commonest and most widespread of all species of *Eumusa*. One probable reason is that it was not until a central collection of living *Musa* species was formed that anybody was in a position to know that the species was widespread, or to realize its importance. Another is the obsession of so many authorities with the idea that any *Musa* related to a banana must be referred to *M. sapientum*. Acceptance of the fact that there can be no true 'wild form' of *M. sapientum* should go far in clearing up the nomenclature of the genus.

REFERENCES

CHEESMAN, E. E., WARDLAW, C. W. & SPENCER, G. L. (1933). The Cavendish group of banana varieties. *Trop. Agriculture, Trin.*, **10**, 218–21.

COLLA, L. (1820). Memoria sul genere *Musa*. *Mem. Accad. Sci. Torino.*

KURZ, S. (1865). Note on the plantains of the Indian Archipelago. *J. Agric.-Hort. Soc. India*, **14**, 295–301.

DESYNAPSIS AND OTHER ABNORMALITIES INDUCED BY HIGH TEMPERATURE*

By W. K. PAO and H. W. LI

(With Twenty-three Text-figures)

Desynapsis in meiotic division has been found in many plants such as *Zea* (Beadle, 1930), *Triticum* (Li, Pao & Li, 1945), *Datura* (Bergner, Cartledge & Blakeslee, 1934), *Pisum* (Koller, 1939), etc. Genetically, this phenomenon is due to a recessive gene, but its intrinsic mechanism is still unknown.

In the course of the study of desynapsis of the common wheat, the writers found that increase of temperature might have some effect on the reduction of univalent frequency in some desynaptic plants (Li *et al.* 1945). This finding stimulated the writers to undertake a series of experiments on high-temperature treatment in order to test how normal plants would be affected.

Material and methods

Triticum vulgare Vill., diploid and tetraploid *Secale cereale* L., *Hordeum vulgare* L., *Vicia cracca* L. and *V. faba* L. were used in the experiments. In the majority of cases only cut stalks were used. The cut stalks, with their lower part immersed in tap water, could maintain growth quite normally for at least 1 week under room conditions. For *V. faba* the whole plant transplanted in small plots was used. After treatment they were transplanted back to the field.

The temperature used in the experiments varied from 25 to 45° C. From lack of good equipment, the incubator was quite poor, so it was found impossible to maintain a stable temperature, the variation being ±3° C.

The duration of the treatment varied from ½ to 24 hr. A part of the material was fixed immediately after treatment in acetic-alcohol mixture (1:3) and others were fixed at different intervals, the longest being 9 days. After 1 day the materials were transferred from fixative to 70% alcohol, the aceto-carmine smear method being used exclusively.

Results

Triticum vulgare Vill.

The experiments were carried out in March 1943 and 1944. The average temperature of that month as recorded by a nearby meteorological station was quite constant for each year, usually between 13 and 14° C. The maximum might reach 25·6° C. and minimum 5·5° C.

More than one variety was used in the treatment. The lines 1024, 1025, 2008 and 2012 are of varietal hybrid origin.

No apparent effect of high temperature could be found at early prophase of the first meiotic division, and individual chromosome threads were clearly observed. Although no attempt was made to scrutinize the pairing condition of the chromonemata, yet from the

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presence of chiasmata of the bivalents at diplotene it is legitimately inferred that the pairing of homologous chromosomes at early prophase might have taken place.

The immediate effect of high temperature (30–40° C.) became apparent at diakinesis. The chromosomes were highly contracted and nearly all of the chiasmata were terminalized

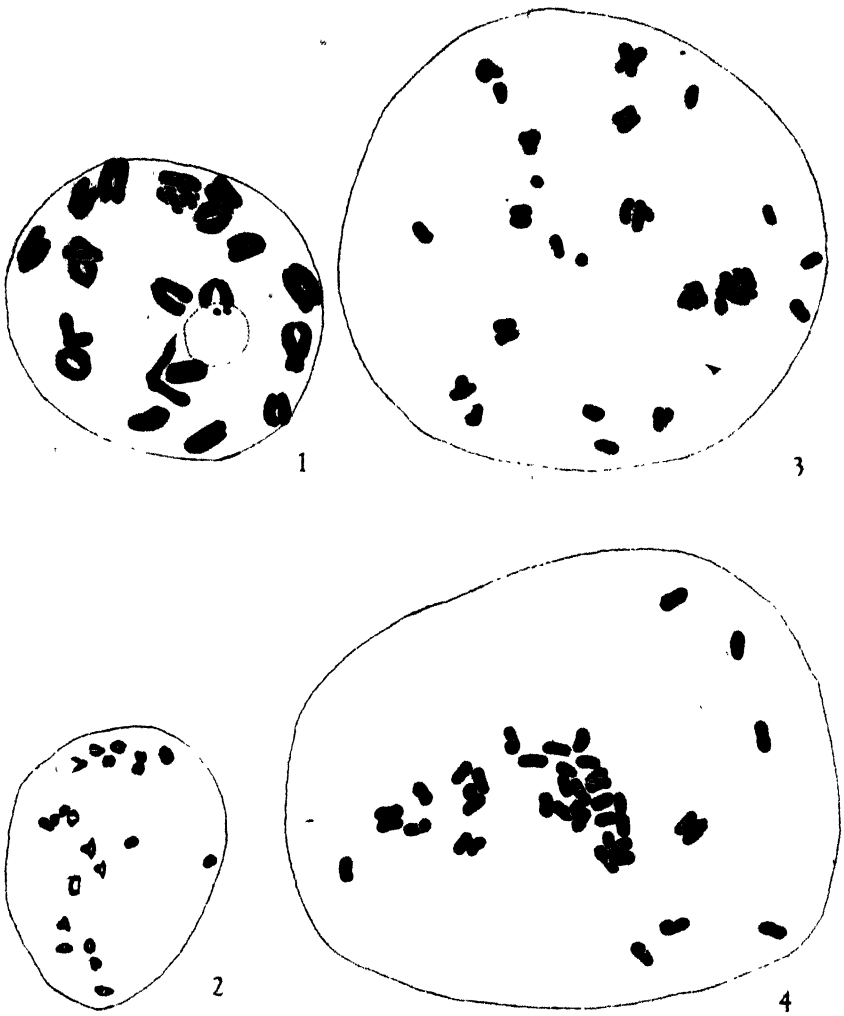


Fig. 1. Line no. 2012. 35° C. for 10 hours. Diakinesis showing the contraction of chromosomes and the terminalization of chiasmata. × 1700.

Fig. 2. Line no. 2008. 40° C. for 2 hours. Metaphase showing the random orientation of the bivalents. × 760.

Fig. 3. Line no. 1024. 32° C. for 24 hours, showing the haphazard aggregation of the chromosomes of a seriously affected cell. × 1700.

Fig. 4. Line no. 1024. 32° C. for 24 hours. A cell with 42 univalents. × 1700.

at this stage (Fig. 1). Moreover, in some cells few univalents could be found. The complete terminalization is probably due to the excessive contraction of the chromosomes, while in the normal cell of an untreated plant the terminalization of chiasmata was never complete until the beginning of anaphase.

At metaphase it was found that no regular equatorial plate was formed. The bivalents

were scattered all over the cell, as were the univalents, and no regular spindle region could be observed. There was no definite orientation of the centromeres in relation to the poles, and the bivalents might take any angle in relation to the pole axis (Fig. 2). In the majority of cases, separation of homologous chromosomes of the bivalents did not take place at anaphase, but instead they had a tendency to move to the periphery of the cell and consequently aggregated into groups of various numbers. Thus more than two daughter nuclei might be formed after the first division.

A few cells seemed to be affected more seriously. Univalents intermingled with bivalents were scattered haphazardly all over the cell. Apparently the chromosomes were paralysed, for they had no tendency to move around as in the normal cells. The chromosomes in juxtaposition to each other, however, tended to aggregate together to form micronuclei later on (Fig. 3). Cells with 42 univalents were found (Fig. 4), but such cases were extremely rare.

In the second division, the repulsion of the sister chromosomes of the dyads became obvious at late prophase. Invariably, each dyad took a cross form with the undivided centromere as its cross-centre (Fig. 5), but this is seldom the case in the untreated material. The centromeres, however, might divide at late prophase, and consequently the separation of the sister chromatids at this stage became complete, as a result of which 42 monads would be included in each nucleus (Fig. 6). It was noted, however, that the division of the centromere might be only for some of the dyads but not for others. As a result, nuclei with a mixture of dyads and monads could be found.

At the second metaphase a similar irregularity was found as in the first metaphase. The dyads did not congress regularly to form an equatorial plate; instead, they scattered around all over the cell (Fig. 7). The centromeres might remain undivided even at late anaphase (Fig. 8), and consequently, micronuclei were frequently found.

When the temperature was raised higher than 40° C., the chromosomes became clumped together and their individualities might be lost completely. At metaphase, only a single large chromosomal clump with irregularly outlined margin could be found, while at anaphase many small oil-drop-like masses might make their appearance. These phenomena were observed both in the first and the second divisions. Similar cases were found in some untreated material which was fixed on a fine afternoon. Fig. 9 shows the oily condition of chromosomes at second anaphase of an untreated plant (from a line selected from the cross between variety Quality and pure line 18–3874, 1942). This phenomenon is in no way comparable to the 'sticky' *Zea* (Beadle, 1937). It had been considered at first to be due to some new mutation, but in the second fixation of these plants no such abnormality could be found. After comparing the results obtained in the experiments of temperature treatment, it was apparent that it might be the result of the local effect of high temperature on those spikes which were being exposed to the strong sunlight.

Secale cereale L.

The experiment was carried out on 4–8 April 1944. The maximum field temperature during these days was 27·5° C. and the minimum 15·5° C. Both diploid and tetraploid rye were used. Untreated stalks of both kinds of rye, cultured in tap water under the room condition at the beginning of the experiment and fixed on 7 April, were used as checks. The temperature used for treatment was 36° C. and the duration of the treatment was 24 hr. The first fixation was made at 24 hr. after the end of heat treatment, and two other fixations were made at successive periods at 24 hr. intervals.

Diploid rye. The diploid rye has 14 somatic chromosomes. Seven bivalents are regularly formed at meiosis. The only abnormality found in this treatment was the appearance of

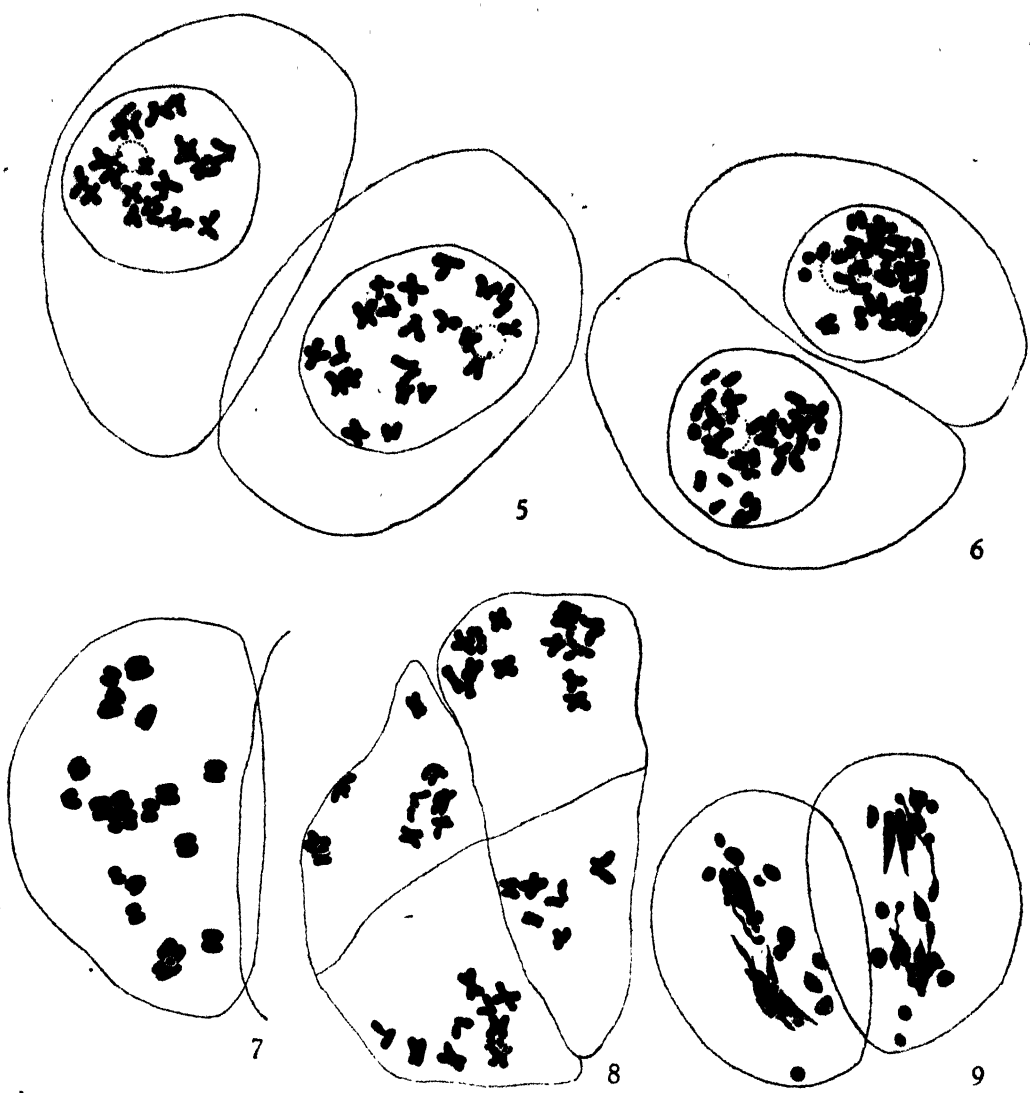


Fig. 5. Line no. 1024. 32° C. for 24 hours. Second division, late prophase; each dyad takes a cross form with the undivided centromere as its cross-centre. × 1330.

Fig. 6. Line no. 1024. 32° C. for 24 hours. Second division, late prophase; each nucleus includes 42 monads. × 1330.

Fig. 7. Line no. 1025. 32° C. for 24 hours. Second division metaphase showing the haphazard orientation of the dyads all over the cell. × 1700.

Fig. 8. Line no. 2012. 35° C. for 6 hours. Second division late anaphase showing the random distribution of the dyads with the centromeres still undivided. × 1700.

Fig. 9. Line no. 1483—12. The figure shows the oily condition at the second anaphase of an untreated plant fixed on a fine afternoon. × 1330.

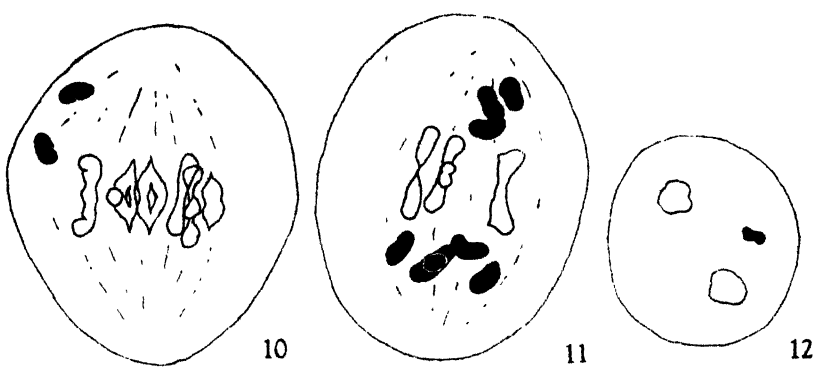
univalents. About 18·8% of pollen mother cells showed the presence of univalents at 24 hr. after treatment. The maximum number of univalents observed was 4, while at 48 hr. after treatment the percentage of cells with univalents increased to 55·1, and the

maximum number of univalents reached was 8 (Table 1). Figs. 10 and 11 show the occurrence of univalents, one with 2 the other 8.

The univalents, as a rule, did not divide at the first division, but went to either pole somewhat randomly at anaphase. From a count of chromosome distribution at the second prophase only one out of twenty-seven cells showed the 0–8 distribution of the first fixation. For the second fixation it was two out of ten cells. The univalents might lag and be excluded from either of the daughter nuclei. Fig. 12 shows the lagging of a univalent. However, the occurrence of lagging univalents was not frequent.

Table 1. *The univalent frequency of the diploid rye after treatment with high temperature* (36° C.) *for* 24 *hr.*

Hr. after treatment	No. of bivalents 0	1	2	3	4	5	6	7	No. of cells counted	% cells with univalents	Stage of cells
24	.	.	.	.	.	2	7	39	48	18·8	Metaphase
48	.	.	.	1	3	11	23	31	69	55·1	Metaphase
48	.	.	.	.	3	4	5	7	19	63·2	Diakinesis
Control					All				—	0	Metaphase



Figs. 10–12. Diploid rye treated at 36° C. for 24 hours. Fig. 10. Fixed 48 hours after the end of treatment. Shows the presence of 2 univalents at first metaphase stage. ×1700. Fig. 11. Fixed 48 hours after the end of treatment. Shows the presence of 8 univalents at the first metaphase stage. ×1700. Fig. 12. Fixed 24 hours after the end of treatment. Shows the lagging of a univalent at first telophase. ×760.

The pollen mother cells of the third fixation, 72 hr. after treatment, were highly degenerated. There were no clear figures found, except the quartets with various numbers of micronuclei. In the controls, however, univalents were not found and the division seemed to be quite normal.

Autotetraploid rye. The autotetraploid rye has twenty-eight somatic chromosomes which at meiosis form bivalents and tetravalents in different proportions. After high-temperature treatment (36° C. for 24 hr.), abnormal meiotic division as found in the treated diploid rye was observed, i.e. the appearance of univalents. About 21 % of the pollen mother cells showed the occurrence of two univalents 24 hr. after treatment (Table 2). More than two univalents were not found in this fixation. It was apparent, though, that the occurrence of univalents was not so significant as the reduction of the number of tetravalents, as shown in Table 3. The cells with tetravalents decreased to 53·5 % as compared with 94·2 % in the control, and the maximum number of tetravalents per cell fell from 7 to 2 (Table 3). With fixation 48 hr. after treatment, the number of univalents increased tremendously in each pollen mother cell. Indeed, no cell could be found without univalents. Three cells were

observed to have no bivalent. Fig. 13 shows the most frequent type of cell, with 6 bivalents and 16 univalents. The univalents never split at first division. The separation of the univalents was quite random. This can be seen in Table 4. The expected frequency distribution of univalents was calculated from the univalent frequency in Table 2 (48 hr. after treatment) with a random basis for univalent separation. Although the number of cells counted is not large enough the frequency shows a general tendency to approach the expected one. Tetravalents vanished entirely in the pollen mother cells 48 hr. after treatment.

Table 2. *The univalent frequency of the autotetraploid rye after treatment with high temperature* (36° C.) *for* 24 *hr.*

Hr. after treatment	No. of bivalents 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	No. of cells counted	% cells with univalents	Stage of cells
24	.	.	.	.	.	.	.	.	.	.	.	.	.	7	26	33	21·2	Metaphase
48	3	5	8	11	19	22	28	23	19	11	5	1	.	.	.	155	100	Metaphase
Control	.	.	.	.	.	.	.	.	.	.	.	.	.	.	All		0	Metaphase

Table 3. *The tetravalent frequency of the autotetraploid rye after treatment with high temperature* (36° C.) *for* 24 *hr.*

Hr. after treatment	No. of tetravalents 0	1	2	3	4	5	6	7	No. of cells counted	% cells with tetravalents	Stage of cells
24	7	5	3	.	.	.	.	.	15	53·3	Diakinesis
48	All	.	.	.	.	.	.	.	—	0	Diakinesis
Control	4	18	16	18	6	5	1	1	69	94·2	Diakinesis

Table 4. *The distribution of univalents of the autotetraploid rye after treatment with high temperature* (36° C.) *for* 24 *hr.*

Hr. after treatment	No. of chromosomes of the two daughter nuclei 4–24	5–23	6–22	7–21	8–20	9–19	10–18	11–17	12–16	13–15	14–14	No. of cells counted	% 14–14 type	Stage of cells
48	1	2	1	1	1	2	6	3	6	15	16	54	29·6	1st telophase and 2nd prophase
Control	.	.	.	.	.	.	.	.	2	5	57	64	89·1	1st telophase and 2nd prophase
48 (Expected)	0	0·01	0·02	0·08	0·31	1·06	3·01	6·97	12·94	18·88	10·72	54	19·9	

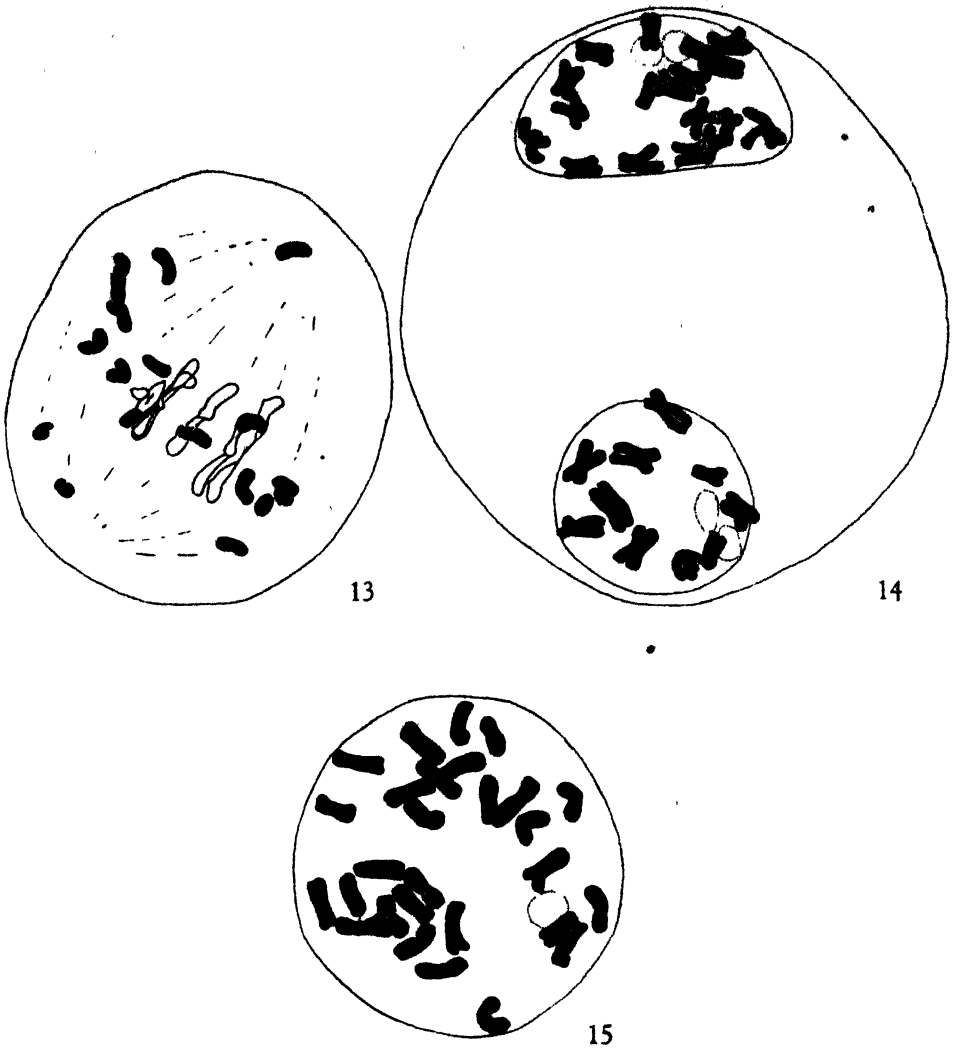
Fig. 14 shows a pollen mother cell at second prophase with the chromosome distribution of 10–18.

Restitutional nuclei were rarely found. Fig. 15 shows such a nucleus at the second prophase. Such cells might be easily confused with those cells in which 28 univalents occurred at diakinesis. However, the univalents never split at diakinesis, while at second prophase most of them began to do so. On this criterion the two can be differentiated.

Hordeum vulgare L.

Cut stalks of cultivated barley, *H. vulgare*, were treated at a temperature of 35° C. for 10 hr. Fixations were made immediately after the treatment. The immediate effect of the high temperature was that the chromosomes became highly contracted and nearly all of the chiasmata were terminalized at diakinesis. No univalents, however, were found at diakinesis or metaphase. At metaphase a regular equatorial plate could not be observed, and the bivalents were scattered all over the cell as in common wheat after heat treatment; nor was a spindle region formed. At anaphase the bivalents in juxtaposition to one another seemed to group together; thus, one to three groups might be formed (Figs. 16–18). The frequencies of bivalent distribution in these groups are shown in Table 5.

From the data it is apparent that there are three kinds of cell polarity for the distribution of bivalents, namely, about one-fourth unipolar, one-half bipolar and one-fourth



Figs. 13–15. Tetraploid rye treated at 36° C. for 24 hours and fixed 48 hours after the end of treatment. × 1700. Fig. 13. Shows the occurrence of 16 univalents and 6 bivalents at first metaphase. × 1700. Fig. 14. Shows the distribution of chromosomes at second prophase due to the haphazard behaviour of univalents in the first division. × 1700. Fig. 15. Shows a restitutional nucleus at second late prophase including 28 dyads. × 1700.

Table 5. *The distribution of bivalents at late anaphase of barley treated at* 35° C. *for* 10 *hr.*

	Distribution types of bivalents								
	Unipolar	Bipolar			Tripolar				No. of cells counted
	0–7	1–6	2–5	3–4	1–1–5	1–2–4	1–3–3	2–2–3	
Observed	6	3	4	8	0	1	2	3	27
Expected	6·96	1·61	4·82	8·04	0·39	1·94	1·30	1·94	27

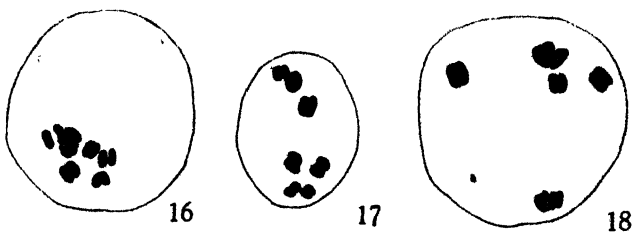
tripolar. Only the 0–7 type of distribution could occur in the unipolar cells, while in the bipolar cells the distribution of chromosomes might be expected to follow the expansion of the binomial $(1+1)^7$. For the tripolar cells the theoretical distribution of chromosomes

might be calculated from the expansion of the trinomial $(1+1+1)^7$. Thus the expected frequency in Table 5 was calculated, and it fits the observed very well.

No division was observed at telophase of these randomly distributed bivalents. The after-effect of this meiotic abnormality was not traced to the second division for no successive fixations were made.

Vicia cracca L.

The common vetch cultivated in the Chengtu plain belongs to the species *V. cracca*. The experiments were carried out on 14 and 26 January 1943. The field temperature for both days when the stalks were taken for experiments was 7° C. Three temperatures, 25, 35 and 45° C., were used for treatment. Univalents made their appearance after treatment for 30 min. The cells with univalents seemed to increase with the increase of the temperature,



Figs. 16–18. Barley treated at 35° C. for 10 hours and fixed immediately after treatment. The figures show the three kinds of cell polarity for the distribution of bivalents. Fig. 16. Unipolar. Fig. 17. Bipolar. Fig. 18. Tripolar. ×760.

namely, 50% at 25° C., 57·1% at 35° C. and 60% at 45° C. (Table 6). The increase of univalents is even more marked if the duration of treatment is prolonged. After treatment for 75 min. the cells with univalents increased to 86·1% at 25° C. and 88·9% at 35° C. With incubation at 35° C. for 15 hr. univalents appeared in every cell observed and the majority (about 78%) had no bivalents at all (Fig. 21). Univalents were not found in the untreated pollen mother cells.

Table 6. *The univalent frequency in the common vetch due to high temperatures*

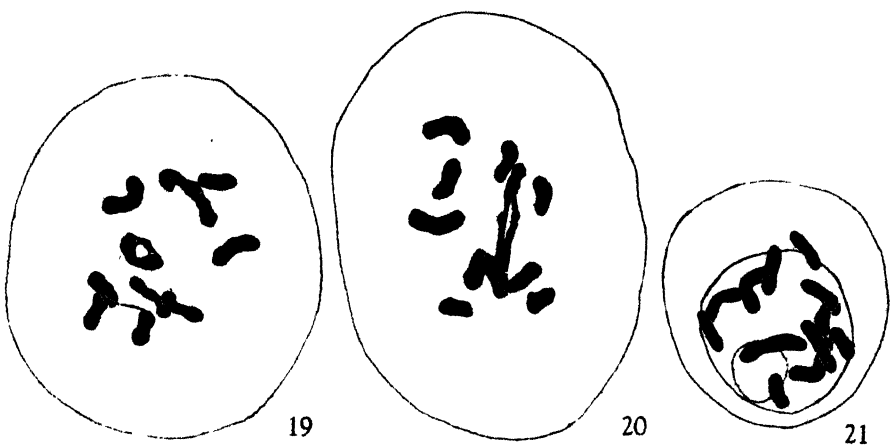
Temp. used in treatment ° C.	Duration of treatment	Date of treatment	No. of bivalents 0	1	2	3	4	5	6	No. of cells counted	% cells with univalents	Stage of cells
25	30 min.	26. i. 43	.	.	.	.	2	1	3	6	50·0	Metaphase
25	75 min.	26. i. 43	.	.	1	2	8	2	2	15	86·1	Metaphase
25	9 hr.	14. i. 43	1	3	4	5	1	3	2	19	89·5	Metaphase
35	30 min.	26. i. 43	.	.	.	.	1	3	3	7	57·1	Metaphase
35	75 min.	26. i. 43	.	.	.	1	2	5	1	9	88·9	Metaphase
35	15 hr.	14. i. 43	74	15	5	1	.	.	.	95	100·0	Metaphase and diakinesis
45	30 min.	26. i. 43	.	.	.	.	1	2	2	5	60·0	Metaphase

Vicia faba L.

Two series of experiments were carried out, one in January 1943, together with those on the common vetches, the other in February 1944. In the first series of experiments only cut stalks were used for treatment and no univalents were found in the pollen mother cells which were fixed immediately after treatment with temperatures from 25 to 45° C. for a period of time from 30 to 110 min. The result was thus quite different from that of the common vetches just described. In the second series of experiments plants transplanted in small pots from the field were used for treatment. After treatment the plants were

transplanted back again to the field. The fixations were made from 1–9 days after treatment at different high temperatures for 24 hr. The univalents usually made their appearance 1 day after treatment, but the maximum frequency could be reached 2–4 days after treatment. The appearance of univalents might last for 9 days after treatment, but their frequency decreased gradually after the maximum was reached (Table 8). Three days after treatment at 45° C. for 24 hr., not a single bivalent could be found in ninety-six pollen mother cells observed.

The somatic chromosome number of *V. faba* is 12, one pair of which is nearly twice the size of the other five pairs of approximately equal size. It may be expected that the chance for the appearance of univalents for the larger pair would be lower than for the smaller pairs if the formation of univalents is due to the complete terminalization of chiasmata. Actually such was the fact found. As shown by the lower part of Table 8, the mean



Figs. 19, 20. Common vetches, treated at 25° C. for 9 hours, showing the metaphase with 4 and 10 univalents.
Fig. 21. Common vetches, treated at 35° C. for 15 hours, showing a pollen mother cell at diakinesis stage with 12 univalents. × 1700.

univalent formation per bivalent for 1 day after treatment at 35° C. was 78·6 %; it was 89·6 % 4 days after; and it was 31·8 % for a plant grown under natural summer conditions. When the large bivalent was excluded, they increased to 85·6, 95·0 and 35·5 % respectively. On the other hand, when the large chromosome was considered alone, the corresponding univalent formations were 43·8, 62·5 and 13·6 % respectively. These are relatively much lower in comparison with the average for the other five pairs.

The number of chiasmata per pollen mother cell was counted in testing whether high temperature has any effect upon the terminalization of the chiasmata. However, no immediate effect was found after treatment for 30–110 min. at 25, 35 and 45° C. (Table 7). But the after-effect of heat treatment was significant. The number of chiasmata fell concordantly with the appearance of univalents and reached a minimum at 3 days after treatment when the number of univalents was at a maximum. Thereafter, however, the chiasmata per cell increased in company with the decrease of univalent frequency. At 9 days after treatment an approximately normal condition was regained.

Micronuclei were frequently found at the quartet stage. This shows that lagging of these artificially induced univalents was not uncommon.

Table 7. *Chiasma frequency of the broad bean treated with high temperatures*

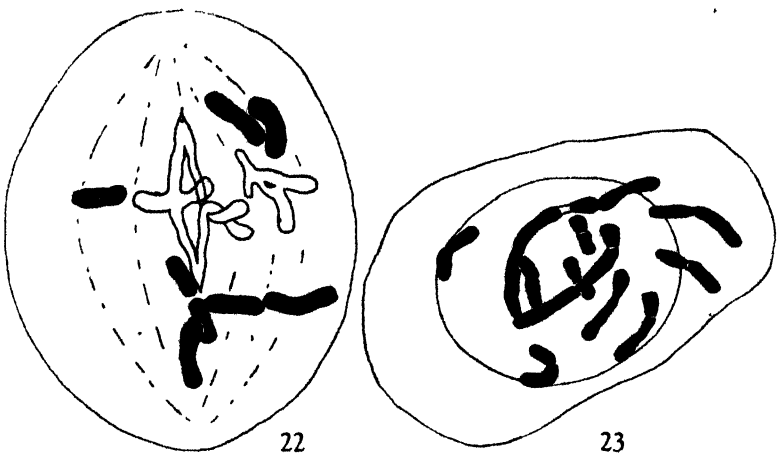
Temp. used in treatment ° C.	Duration of treatment	Date of fixation	Days after treatment	Chiasma frequency 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	No. of cells counted	Mean no. chiasmata
7·5 (control)		26. i. 43	—	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	0	1	1	3	5	0	0	1	12	20·2
25	30 min.	26. i. 43	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	0	2	4	2	2	0	11	21·1
25	75 min.	26. i. 43	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	0	1	4	4	0	2	0	12	20·5
35	30 min.	26. i. 43	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	1	4	4	3	1	1	16	20·8
35	75 min.	26. i. 43	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	1	2	4	3	1	.	.	12	19·8
35	110 min.	26. i. 43	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	1	2	1	4	1	.	.	10	19·9
45	30 min.	26. i. 43	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	4	3	5	5	3	.	.	23	19·6
45	75 min.	26. i. 43	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	6	3	4	.	.	.	14	19·7
45	110 min.	26. i. 43	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	0	1	3	0	0	1	.	.	6	18·8
9 (control)		15. ii. 43	—	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	0	2	4	2	3	12	22·3
35	24 hr.	28. ii. 43	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	0	0	0	2	2	1	5	2	1	14	21·0
35	24 hr.	29. ii. 43	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	2	0	1	4	1	1	.	.	.	10	18·2
35	24 hr.	1. iii. 43	2	21	5	2	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	31	0·6
40	24 hr.	12. ii. 43	3	119	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	125	0·05
40	24 hr.	15. ii. 43	6	1	2	1	4	0	0	3	2	5	1	5	1	5	2	1	3	.	.	.	.	.	.	.	.	.	36	8·4
40	24 hr.	18. ii. 43	9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	1	4	1	3	4	4	2	3	.	.	26	18·1
45	24 hr.	11. ii. 43	3	96	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	96	0·0
45	24 hr.	14. ii. 43	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	3	1	1	2	0	2	.	.	.	10	17·8
45	24 hr.	17. ii. 43	9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	5	6	2	1	.	.	16	19·7

Table 8. *Univalent frequency of the broad bean treated with high temperatures*

Temp. used in treatment ° C.	Duration of treatment	Date of fixation	Days after treatment	No. of bivalents 0	1	2	3	4	5	6	No. of cells counted	% cells with univalents	Stage of the cells
35	24 hr.	28. ii. 43	0	.	.	.	.	.	.	164	164	0	Diakinesis and metaphase (in all cases)
35	24 hr.	29. ii. 43	1	.	.	.	.	.	.	121	121	0	
35	24 hr.	1. iii. 43	2	62	8	1	.	.	.	.	71	100	
40	24 hr.	29. ii. 43	0	3	8	3	8	5	4	12	43	72·4	
40	24 hr.	12. ii. 43	3	119	6	.	.	.	.	.	125	100	
40	24 hr.	15. ii. 43	6	1	2	2	5	11	25	13	59	78·0	
40	24 hr.	18. ii. 43	9	.	.	.	.	1	6	124	131	5·3	
45	24 hr.	11. ii. 43	3	96	.	.	.	.	.	.	96	100	
45	24 hr.	14. ii. 43	6	.	.	.	.	4	5	127	136	6·6	
45	24 hr.	17. ii. 43	9	.	.	.	.	.	.	106	106	0	
35	24 hr.	4. ii. 43	1	12	10	4	4	0	1	1	32	96·9	
35	24 hr.	7. ii. 43	4	24	11	1	4	.	.	.	40	100	
35*	24 hr.	4. ii. 43	1	14	18	.	.	.	.	.	32	43·8	
35*	24 hr.	7. ii. 43	4	25	15	.	.	.	.	.	40	62·5	
Summer day	—	3. viii. 43	—	1	2	1	2	5	6	5	22	77·3	
Summer day*	—	3. viii. 43	—	3	19	.	.	.	.	.	22	13·6	

* For the large chromosome alone.

A few commercial seeds were sown in June 1944. Such plants were slender, devoid of purple stripes on the stems, and the leaves were pale green in colour. The flowers appeared in early August and were colourless except for the characteristic broad black spot on the keels. Another batch of such seeds was grown in the winter. There were plenty of purple stripes which made both the plant and flowers purple in appearance. Moreover, only one single plant was fixed on 3 August. The mean temperature of the day was 32° C. Very much as with the heat-treated plants, univalents were found in the pollen mother cells of this plant, and its frequency is shown in Table 8.



Figs. 22, 23. Broad bean treated at 40° C. for 24 hours. Fig. 22. Fixed 6 days after the end of treatment. First metaphase with 8 univalents and 2 bivalents. Fig. 23. Fixed 3 days after the end of treatment. Diakinesis stage with 12 univalents and no bivalent. It can be seen that the homologous univalents still remain closely together. From this fact it is inferred that pairing of the homologous chromosomes had taken place at the early prophase but the exchange of parts (crossing-over) was prevented. × 1700.

CONCLUSION AND DISCUSSION

From the foregoing description of the abnormalities induced by high temperature it is obvious that the effect of heat upon the meiosis may be divided into two categories, namely, direct effect and after-effect. For the direct effect of high temperature, the most evident fact is the disturbance of the spindle mechanism. The polarity of the cell is seemingly destroyed by the heat and consequently no regular spindle region can be formed. However, the destruction of cell polarity may be, on the contrary, due to the disturbance by heat of the definite orientation of the long spindle-forming molecules (Darlington, 1937), and perhaps comparable with heat disturbance of molecular orientation in crystal formation. But whether the polarity is due to the regular orientation of such long molecules is not definitely known.

With no regular spindle formation, definite orientation of the metaphase chromosomes on an equatorial plate becomes impossible. Both at first and second metaphases the bivalents or dyads scatter somewhat randomly over the cell, a fact very similar to the behaviour of univalents in an interspecific hybrid of different genoms. More curious is the fact that both the bivalents and dyads behave as individual entities and their components remain unseparated even at telophase. In such cases the action of the centromere seems to be paralysed by heat. Consequently, no apparent repulsive force between centromeres can be developed, or else this force is not strong enough to overcome the resistance offered by the chiasmata of the bivalent. Furthermore, the division of the centromere is

prevented even in the second division. The prevention of centromere division at second meiotic division may cause the appearance of diplochromosomes (Barber, 1940) in pollen-grain division. Unfortunately, in this investigation, no pollen-grain division was ever studied.

However, such a paralysing effect on the centromeres is by no means universal for all the pollen mother cells observed even if they come from the same anther. For in some cells the centromeres of the dyads, instead of being paralysed, are divided, precociously at second prophase, and monads appear as shown in Fig. 6. Similarly, few univalents are found at diakinesis stages of the first division. Probably this is the result of the immediate effect of heat. However, there are no signs of strong repulsion between the centromeres of the homologous chromosomes even though they are separated, for they usually remain closely together. Without the strong repulsion between the homologues, the complete terminalization of chiasmata will become very difficult or even impossible. It is, then, evident that the appearance of univalents must be due to another cause which will be discussed in the later paragraphs.

The contradiction of the results may be explained by the non-homogeneity of heat flow and the differences in susceptibility of the chromosomes at different stages of the division cycle. The position of cells in an anther, and the heterogeneity of the tissues, may cause differences of heat energy acquired by the different pollen mother cells and thus different effects will result. An excessive heat flow may paralyse some activities, but a moderate flow may, on the contrary, supply the necessary energy to accelerate the activities. As mentioned before, the prophase is the most resistant stage to heat, while the metaphase stage seems to be the most susceptible. The activities of the components of a division cycle attain their maximum at metaphase when most of the forces are delicately balanced, so that from the effect of heat, the spindle is distorted at this stage. At the same time the centromere is paralysed and the bivalent chromosomes, in turn, after losing their balance, take up a haphazard position at this stage. The abnormalities found at anaphase and telophase can be easily traced to their origin at metaphase.

Quite unexpectedly, heat has no immediate effect upon the terminalization of the chiasmata of such pollen mother cells when their chromosomes are not highly contracted, as, most notably, in the case of *Vicia faba*. Consequently univalents will not appear, due to the immediate effect of heat treatment. Unexpectedly, again, the appearance of univalents is due to an after-effect. The lapse of time from treatment to the appearance of univalents is by no means uniform among the species tested. It also differs with the temperature used. The univalent frequency reaches a maximum about 2 days after treatment in the diploid and tetraploid ryes, 3 days in *V. faba*, and almost immediately in *V. cracca*. In common wheat the occurrence of univalents is quite haphazard and rare, so that no definite relation can be found. No attempt was made to study the appearance of univalents in the barley. In *V. faba* such an after-effect can last as long as 9 days.

An attempt was made to test whether the formation of univalents is due to the non-pairing of homologous chromosomes or to the acceleration of the terminalization of chiasmata, but at present no definite conclusion can be drawn. Counting shows (Table 7) that the number of chiasmata per cell decreases abruptly with the appearance of univalents. It seems that terminalization of chiasmata is the cause of univalent formation. But after a close study on the prophase it was found that univalents may appear at diplotene stage. Complete terminalization at such an early stage seems impossible. An alternative explana-

tion is the non-pairing of homologous chromosomes. However, as has already been stated, the homologous univalents may still remain closely together at late prophase stages (Fig. 23). This fact clearly indicates that pairing of the homologous chromosomes is actually taking place at the early prophase. Therefore, most probably, neither of these alternatives is to be regarded as the true cause of the occurrence of univalents.

The appearance of univalents as an after-effect of high-temperature treatment suggests that the effect is due to the insufficiency or excess of some substance, irrespective of its chemical nature, which has some influence upon the exchange of parts between the homologous chromosomes. In other words, the effect is chemical rather than physical. Crossing-over may thus be hindered, partially or wholly, and the chiasmata will be reduced accordingly. When crossing-over is completely hindered for a particular pair of chromosomes, two univalents will result in early prophase. If the hindrance is not complete for some other pairs, only few chiasmata per bivalent will be formed. These few chiasmata are easily terminalized at diakinesis or at early metaphase, and univalents may thus be formed at these stages.

Although its chemical nature is unknown, the evidence shows that this substance may be affected by heat, controlled by genes (desynaptic gene) and influenced by sex (complete suppression of crossing-over in male *Drosophila* and in female *Bombyx mori*). Moreover, in *Hieracium* it is also related to the tapetum development (Gentcheff & Gustafsson, 1940). Gentcheff & Gustafsson found univalents in young flowers in the middle part of the head of *H. robustum* and *H. amplexicaule*. This type of division (division type 1), called semi-heterotypic, is related to the development of the tapetum. When the tapetum cells are old (with four single or fused nuclei) the meiotic division of the pollen mother cells becomes pseudohomotypic (division type 2), i.e. all univalents are split. The pseudohomotypic division may be regarded as the advanced type of semi-heterotypic division. Matsuura & Haga (1940) treated *Trillium kamtschaticum* at 20° C. for several weeks and found univalents, separated chromatids and half-chromatids in the treated pollen mother cells. All these phenomena, whether induced by artificial means or occurring naturally, seem to belong to the same category of reaction but with different degrees of expression.

SUMMARY

Triticum vulgare, diploid and tetraploid *Secale cereale*, *Hordeum vulgare*, *Vicia faba* and *V. cracca* were used in high-temperature treatment. The temperature used varied from 25 to 45° C. The duration of treatment covered a range from ½ to 24 hr.

The abnormalities in meiotic division induced by the heat were classified into direct effect and after-effect. In the direct effect the spindle formation was at first affected. No regular spindle region was formed, and the bivalents scattered randomly in the cell as the univalents. The centromeres of chromosomes were paralysed. No disjunction of bivalents took place, and consequently one, two or three groups of bivalents were formed haphazardly at telophase. Similar results were observed for the dyads in the second division. The disturbance of spindle formation was explained as probably due to the prevention of definite orientation of the long spindle molecules.

The appearance of univalents was due to the after-effect of heat. The effect reached its maximum about 2 days after treatment for the diploid and tetraploid ryes and 3 days for *V. faba*. For *V. cracca* the effect was almost immediate. The separation of univalents was random. Micronuclei were frequently met with at the quartet stage. The formation of

univalents was explained as due to the prevention of crossing-over between the homologous chromosomes at early prophase, and the prevention of crossing-over seemed in turn to be due to insufficiency or excess of some substance.

REFERENCES

BARBER, H. N. (1940). The suppression of meiosis and the origin of diplochromosomes. *Proc. Roy. Soc.* B, **128**, 170–85.

BEADLE, G. W. (1930). Genetical and cytological studies of Mendelian asynapsis in *Zea Mays*. *Mem. Cornell Agric. Exp. Sta.* no. 129.

BEADLE, G. W. (1937). Chromosome aberration and gene mutation in sticky chromosome plants of *Zea Mays*. *Cytologia, Tokyo*, Fujii Jubilee Vol., **1**, 43–56.

BERGNER, A. D., CARTLEDGE, J. L. & BLAKESLEE, A. F. (1934). Chromosome behaviour due to a gene which prevents metaphase pairing in *Datura*. *Cytologia, Tokyo*, **6**, 19–37.

DARLINGTON, C. D. (1937). The external mechanism of the chromosomes. V. The balance theory of mitosis. *Proc. Roy. Soc.* B, **121**, 310–19.

GENTCHEFF, G. & GUSTAFSSON, A. (1940). The balance system of meiosis in *Hieracium*. *Hereditas, Lund*, **26**, 209–49.

KOLLER, P. C. (1939). Asynapsis in *Pisum sativum*. *J. Genet.* **36**, 275–305.

LI, H. W., PAO, W. K. & LI, C. H. (1945). Desynapsis in the common wheat. *Amer. J. Bot.* **32**, 92–101.

MATSUURA, H. & HAGA, T. (1940). Chromosome studies on *Trillium kamtschaticum* Pall. VIII. On the mitosis-meiosis relationship. *Cytologia, Tokyo*, **10**, 382–9.

THE RELATIONS BETWEEN HETEROPYCNOSIS, SPIRALIZATION AND LAMPBRUSH FORMATION OF THE CHROMOSOMES IN THE SPERMATOGENESIS OF THE ACRIDIDAE

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(With Five Text-Figures)

From a morphological standpoint, the condensation and decondensation of euchromatic chromosomes during the mitotic cycle are mainly due to two factors: the presence or absence of 'kalymma' and the spiralization of the chromonema (Heitz, 1935). Heterochromatic chromosomes, on the other hand, may be permanently condensed. The differential condensation of heterochromatin (heteropycnosis) was formerly considered to be an irreversible phenomenon. However, White (1940) has demonstrated that in the Acrididae the *X*-chromosome may show both 'positive' and 'negative' heteropycnosis at different stages. This state of affairs seems to be somewhat unusual, many species of animals having heterochromatic chromosomes which show only one type of heteropycnosis.

In the growing oocytes of certain fishes, amphibia and birds cytologists have observed another special type of chromosome structure—the so-called lampbrush chromosome (Rückert, 1892, as quoted from Painter, 1940), in which filaments are given off at right angles to the long axis. These filaments have been described as sometimes forming loops; they disappear at the end of the prophase of the first meiotic division.

In the spermatogenesis of certain species of grasshoppers heteropycnosis, coiling of chromonemata, and the formation of lampbrush chromosomes can all be observed. The author has studied these phenomena in testis smears of two species of short-horned grasshoppers, *Phlaeoba infumata* (Oedipodinae) and *Catantops humilis* (Catantopinae). The preparations were stained with aceto-carmine and by the Feulgen method. At pachytene each chromosome was seen to be furnished with numerous filaments attached to the main strand (Fig. 1). Some of these filaments appeared to be of uniform thickness throughout, while others had a terminal thickening. As diplotene approached the filaments increased in length (Fig. 2), although the chromosome as a whole had shortened in the meantime. This increase in the length of the filaments ceased in diakinesis; and at the same time their number appeared to be reduced, as compared with pachytene (Fig. 3*a*, *b*). During the whole of this time no coiling, either of the main chromosome threads or of the lateral filaments, was observed. We shall term the chromosomes with lateral filaments, which were seen in the spermatogenesis of these grasshoppers, lampbrush chromosomes.

During prometaphase a sudden change takes place in the appearance of the chromosomes. At the end of diakinesis the chromonemata begin to spiralize, and the filaments attached to them rotate so that they are no longer regularly arranged along the main axis. At the same time the filaments shorten, so that the outline of the bivalents becomes gradually smoother (Fig. 4). In Feulgen preparations the metaphase chromosomes stain deeply as rather compact bodies, but in aceto-carmine smears one can see a deeply stained sheath

enveloping each chromosome, within which thinner strands can still be traced, accompanied by a few typical filaments radiating from them towards the sheath (Fig. 5).

These changes are seen in all the autosomes. The X-chromosomes of the two species studied were, like those of most Saltatoria, heteropycnotic, heavily stained bodies throughout the meiotic prophase.

Fig. 1. Part of an autosomal bivalent in pachytene (× 2500).

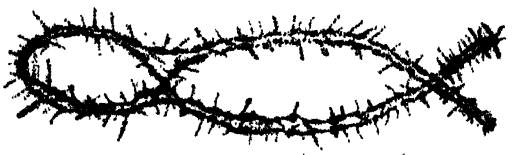

Fig. 2. A diplotene autosomal bivalent (× 2500).

Fig. 3*a*. Diakinesis, showing lampbrush autosomes and compact X-chromosome (× 900).

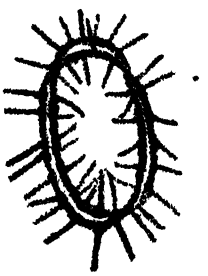

Fig. 3*b*. Diakinesis, showing a single autosomal bivalent (× 2500).

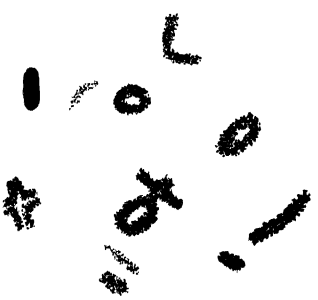

Fig. 4. Prometaphase (× 900).

Fig. 5. An autosomal bivalent in first metaphase (× 2500).

Discussion

In selachian and amphibian oocytes the lampbrush chromosomes appear first at pachytene and become most distinct towards the end of this stage. In diplotene and diakinesis the filaments gradually diminish in number, and the chromosomes lose their fuzzy appearance before diakinesis. In grasshopper spermatocytes, on the other hand, the development of the lampbrush chromosomes proceeds continuously throughout the whole of the meiotic prophase and reaches a maximum at diakinesis. Moreover, the filaments of the lampbrush chromosomes in vertebrate oocytes usually form loops, whereas those of the grasshopper spermatocyte chromosomes are quite straight. Apart from these differences, however, these two types of lampbrush chromosome seem to be alike in principle.

Rückert (l.c.) was probably the first to put forward the idea of the polymeric nature of lampbrush chromosomes such as those of *Pristiurus* oocytes. He claimed that the filaments of such chromosomes were composed of chromatic material and could be considered as single chromosomes. There has recently been much discussion of the polymeric hypothesis of chromosome structure. In the nurse cells of *Drosophila*, Painter & Reindorp (1939) have produced evidence that each chromosome is multiple in nature and is derived from the repeated intranuclear division of an originally single chromosome, thus forming a hairy-caterpillar-like aggregate strikingly similar to a lampbrush chromosome. Salivary gland chromosomes in dipterous larvae may be considered as chromosomes in which a great number of identical protein fibres lie parallel to one another, forming a giant bundle. If such a chromosome is treated with strong alkali a characteristic change in structure occurs (Calvin, Kodani & Goldschmidt, 1940; Calvin & Kodani, 1941), leading to a 'lampbrush' appearance. The nature of this change is not known, but it seems to involve a change in positional relation among the fibres, from a parallel arrangement to a lampbrush condition. These examples are sufficient to indicate that the formation of highly polymeric chromosomes is a common phenomenon in many animal cells of large size.

The polymeric theory of lampbrush chromosomes was definitely accepted by Painter (1940), and on the basis of our own observations we accept his view. Since the component parts of lampbrush chromosomes do not show any spiral structure in prophase, the intense staining of these chromosomes by aceto-carmine and by the Feulgen method must be due to the high concentration of chromatic material, a fact which is comprehensible only if the lampbrush chromosomes are highly polymeric. Again, the increase in length of each filament during diakinesis is more than ten times as great as that at pachytene, and this increase is not comparable to the decrease in length of the main strands during the same period. But, due to the absence of coiling in the lampbrush state, this change in length is very difficult to interpret. Nevertheless, we think it may be somehow related to a yet undefined mechanism involving folding and stretching of the component parts within the polymeric bundle.

The discovery that the *X*-chromosomes of short-horned grasshoppers show a reversible cycle of condensation (White, 1940) threw new light on the problem of heteropycnosis. In early spermatogonial divisions, the *X*-chromosomes show negative heteropycnosis (under-condensation in the sense of Darlington, 1937), i.e. they are less condensed than the autosomes. In late spermatogonial divisions the *X*-chromosomes behave more and more like the autosomes, so that no heteropycnosis can be observed. Still later, during the meiotic prophase, the *X*-chromosomes show strong positive heteropycnosis (over-condensation), while the autosomes appear as slender threads.

Having regard to this variation in the extent and reversibility of condensation, chromatin may be classified into three different types: non-heterochromatic (euchromatic), reversibly heterochromatic and non-reversibly heterochromatic. Before the work of White, Heitz (1934) had already distinguished two types of heterochromatin which he called α- and β-heterochromatin. Neither of these types showed any visible differentiation of chromomere structure, but the β-heterochromatin, like euchromatin, possessed the ability to grow. It is probable that Heitz's β-heterochromatin can be compared to the reversible heterochromatin. The essential difference between these types of chromatin is unknown as yet, but there is some evidence from ultra-violet microscopy that they have

a different quantity of nucleic acid. The view that the principal mechanism of heteropycnosis of chromatic materials is closely linked to their different powers of nucleic acid synthesis requires further elucidation before being finally accepted. But we believe it may not be the sole factor that can account for the phenomenon, since an intimately coiled chromonema with a considerable amount of kalymma could also look like a compact and deeply staining mass. Further, heterochromatin and euchromatin are always practically indistinguishable in metaphase and anaphase—it is only during prophase and telophase that their differentiation becomes distinct, due to the high solvation power of euchromatin at these stages. Taking all these facts into consideration, it seems reasonable to say that the difference between the three types of chromatin is essentially a difference in capacity to undergo cyclic condensation.

The *X*-chromosomes of grasshoppers are extraordinarily compact, and more deeply stained during the meiotic prophase than the autosomes. White (1940) demonstrated that the spiralization of the *X*-chromosome is detectable in the later spermatogonial divisions, but he failed to show this in the spermatocytes. Not until the work of Coleman (1943) and Sze (1946) was it found that coiling of the *X*-chromosomes occurred in meiosis as well. In our own preparations coiling of the *X*-chromosome in zygotene-pachytene and of the autosomes in the spermatogonia was frequently observed. In earlier spermatogonial divisions spiralization of the *X*-chromosome is absent. As shown in Fig. 6 of White's paper (1935) the negatively heteropycnotic *X*-chromosome during this stage is in all respects a lampbrush chromosome.

To explain the behaviour of the chromosomes during the spermatogenesis of these grasshoppers, we are obliged to assume that both the *X*-chromosomes and the autosomes are highly polymeric. These chromosomes may possess an unusual capacity for undergoing changes of state (spiralization, despiralization and the sending out of side folds at regular intervals along the main strands). Where the intracellular conditions favour the exhibition by the *X* of a lampbrush structure and cause the autosomes to be compactly coiled (as in the early spermatogonial divisions) negative heteropycnosis of the *X* will result. Where the conditions are reversed, as in the meiotic prophase, the *X*-chromosome remains compactly coiled, while the autosomes all show the typical lampbrush structure; under these circumstances the *X* may be described as positively heteropycnotic. During the transition between these two extreme conditions the autosomes and the *X* are both similarly coiled and do not give off any side folds so as to produce a lampbrush appearance. This is what we found during the later spermatogonial divisions and may be called the stage of non-heteropycnosis. As the metaphase of the first meiotic division is reached the hairy autosomes again become spiralized and deeply stained like the *X*-chromosome.

In short, reversible heteropycnosis in the Acrididae may be regarded as the result of an interconversion between spiralization and lampbrush formation, only one of these states being found at any one time. This 'brush-spiral' cycle of *X*-chromosomes and autosomes leads to the complex heteropycnotic behaviour of the *X*-chromosome in the Acrididae. The explanation is only tentative, and we hope that further investigations will yield more valuable information.

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REFERENCES

Calvin, M. & Kodani, M. (1941). The physico-chemical structure of the salivary gland chromosomes. II. *Proc. Nat. Acad. Sci., Wash.*, **27**, 291.

Calvin, M., Kodani, M. & Goldschmidt, R. (1940). Effect of certain chemical treatments on the morphology of salivary gland chromosomes and their interpretation. *Proc. Nat. Acad. Sci., Wash.*, **26**, 340.

Coleman, L. C. (1943). Chromosome structure in the Acrididae with special reference to the *X*-chromosome. *Genetics*, **28**, 2.

Darlington, C. D. (1937). *Recent Advances in Cytology*. London: Churchill.

Heitz, E. (1934). Über α- und β-Heterochromatin sowie Konstanz und Bau der Chromomeren bei *Drosophila*. *Biol. Zbl.* **54**, 588.

Heitz, E. (1935). Chromosomenstruktur und Gene. *Z. indukt. Abstamm.- u. VererbLehre*, **70**, 402.

Painter, T. S. (1940). On the synthesis of cleavage chromosomes. *Proc. Nat. Acad. Sci., Wash.*, **26**, 95.

Painter, T. S. & Reindorp, E. C. (1939). Endomitosis in the nurse cells of the ovary of *Drosophila melanogaster*. *Chromosoma*, **1**, 276.

Sze, L. C. (1946). Cytological studies on Acrididae. IV. The structure of the *X*-chromosome in the meiosis of *Phalaeoba infumata*. *J. Morph.* **79**, 113.

White, M. J. D. (1935). The effects of X-rays on mitosis in the spermatogonial division of *Locusta migratoria* L. *Proc. Roy. Soc.* B, **119**, 61.

White, M. J. D. (1940). The heteropycnosis of sex-chromosomes and its interpretation in terms of spiral structure. *J. Genet.* **40**, 67.

THE CYTOLOGY OF THE SPECIES HYBRID *SALVIA NĚMECII* HRUBÝ

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(With Four Text-figures)

Introduction

The name *Salvia Němecii* was given by the author to the interspecific hybrid obtained in 1932 by artificial pollination of the pontic-oriental species *S. nutans* L. with pollen of the Macedonian species *S. Jurišićii* Koš. A reciprocal cross was also made, and yielded plants quite indistinguishable from those of the first experiment. The Latin diagnosis is given by Hrubý (1935, p. 37).

But the plants resulting from the artificial pollination in 1932 were not the first existing individuals of this hybrid. The first plant occurred spontaneously a year earlier among normal plants of *S. nutans*. From its general appearance I concluded that it was a hybrid between *S. nutans* and *S. Jurišićii*. This opinion was supported by a detailed analysis from the morphological, anatomical and, in part, cytological points of view, which is described in a preliminary report (Hrubý, 1933). In order to ascertain the probable origin of the spontaneous hybrid the species were artificially crossed, and the hybrids so obtained were identical with the original spontaneous individual. This intermediate hybrid was thoroughly described and its anatomical characteristics given in the preliminary report (Hrubý, 1933) and were therefore not repeated in publishing the diagnosis, nor need they be repeated here.

The cytological investigation up to the present covers first of all the somatic chromosomes and the haploid phase at meiosis in both parent species (Hrubý, 1933, 1934). Mitosis and meiosis were also studied in three plants of the segregating F_2, and somatic chromosomes in several F_3 plants. *S. Němecii* is relatively very fertile, and, as already stated (Hrubý, 1933, 1935), its progeny segregates very markedly. Various types occur looking nearly like one or other of the parents, or resembling the intermediate F_1. A detailed Mendelian analysis will be given later. Among F_3 and F_4 plants mutations of flower colour also occurred, that is to say, plants with pink or deep rose-coloured flowers. These will be discussed in a later paper. A plant of strikingly low growth with small and agglomerated flowers also appeared in F_3, and its cytology is here described. Thus the present paper deals with the cytology of the F_1, with some reference to that of later generations.

Materials and methods

The material was already fixed in 1932–4, but it was not examined till 1944 and 1945. The original cultures of experimental plants were almost completely destroyed during the war. We succeeded in keeping a few plants near Prague and in the country, but shall be obliged to start afresh for further experiments. Flower buds were fixed in Nawashin's mixture (1% chromic acid 30 parts, 40% formalin 8 parts, glacial acetic acid 3 parts), then embedded and cut, and slides stained exclusively with Feulgen's nuclear reagent. This method of staining proved to be the most advantageous for reliable demonstration of the most delicate structures during meiosis, especially in its early stages. It has many advantages over other staining methods, for example, Heidenhain's haematoxylin method, especially when studying species and hybrids of the genus *Salvia*. In Salvias, which are gyno-

dioecious plants, the stamens often degenerate more or less progressively even in very good species, and more so in interspecific hybrids. As a rule such flower buds fix very badly and stain still worse, especially with haematoxylin, their differentiation being a difficult task. Moreover, in quite normal flower-bud cells and in the root-tip meristem small particles of tannin and small drops of ethereal oils and perhaps other ergastic substances are found, all of which stain very well, especially with haematoxylin. This makes the observation of chromosomes and their behaviour rather difficult. It is probable that many statements as to the presence of extranuclear chromatin and other abnormalities are due to the use of haematoxylin, which, however, is a very good stain, being much more permanent than, for instance, gentian violet, which is more selective with an appropriate technique, but generally fades quickly. The advantage of Feulgen's nuclear reagent is that it stains permanently only those nuclear particles which contain thymonucleic acid, i.e. the so-called karyotin (formerly chromatin), the main substance of the chromosomes. It is also superior in staining all stages of meiosis, even those which usually stain very slightly or not at all with haematoxylin or gentian violet. It is, however, necessary to try to adjust the time of hydrolysis to the object studied and the meiotic stage concerned. After staining with Feulgen, the slides may also be stained for a short time with light green (0·5–1 % aqueous solution). The observation of such slides is more convenient, and this method has already been used for some years in the Plant Physiology Laboratory of the Charles University (Hrubý, 1937). Recently, Gates (1939) has made double staining with Feulgen and light green available for the study of relations between nucleolar chromosomes (sat.-chromosomes) and the nucleolus, which stains slightly green.

SOMATIC CHROMOSOMES

Somatic chromosomes of the parent species had previously been studied in cells of the root-tip meristem, and those of *S. Němecii* in the integument cells of young ovules, which are sometimes a little smaller than those of the root periblem. But after a careful search, many metaphases can be found in which the chromosomes are sufficiently separated to make it possible not only to count them without difficulty, but even to observe their size and shapes. The number found in both parent species and in the hybrid is 22. The chromosomes in all *Salvia* species from the taxonomic section *Plethiosphace* (to which our plants belong) so far investigated, are relatively very small, having the shapes of little rods either straight or slightly bent. Their average length is 2·5–3 μ; the longest do not reach 4 μ, and the shortest are only 1·5 μ long. Owing to these very small dimensions, we cannot exactly distinguish the different pairs of chromosomes, especially when a large number is present, as not even the centromeres (primary constrictions) are discernible. Thus we can set out idiograms only according to the sizes of the chromosomes, the different pairs of which are marked with Roman numerals. Idiograms of both parent species and of *S. Němecii* are shown in Fig. 1, along with two diploid metaphases of *S. Němecii*, and one tetraploid metaphase of the same plant, discovered in a cell of the tapetum.

The somatic chromosomes of *S. nutans* and *S. Jurišićii* show considerable morphological similarity, as is obvious in the idiograms. In each species there is one distinctly largest pair (I, *c.* 3·7 μ long), one pair (II) a little shorter, six pairs (III–VIII) of medium size, gradually diminishing, and finally three small pairs, the shortest about 1·6 μ long. The same range of sizes is found in their hybrid *S. Němecii*.

The presence of satellites creates a very interesting situation. In *S. Jurišićii* one pair

of chromosomes of medium size (VII) has globular satellites. On the contrary, both *S. nutans* and *S. Němecii* show nuclear asymmetry. In all plants of *S. nutans* placed at our disposal (they came from the Botanic Garden of the Masaryk University in Brno) and cytologically investigated, only one chromosome with a satellite has been found. This is one of the larger chromosomes of medium size (pair III in the idiogram). The satellite is so large that the possibility of overlooking a second one in the 123 cases investigated can be excluded. It seems therefore that in our plants of *S. nutans* we have an instance of nuclear asymmetry, such as is already known in several plant species. The first case coincides with the discovery of the satellites themselves. In 1912 S. G. Nawashin described globular bodies of unequal size connected by threads with two chromosomes in *Galtonia candicans*. Later S. G. Nawashin (1927) found only one satellite in *Muscari tenuiflorum*, and after this several

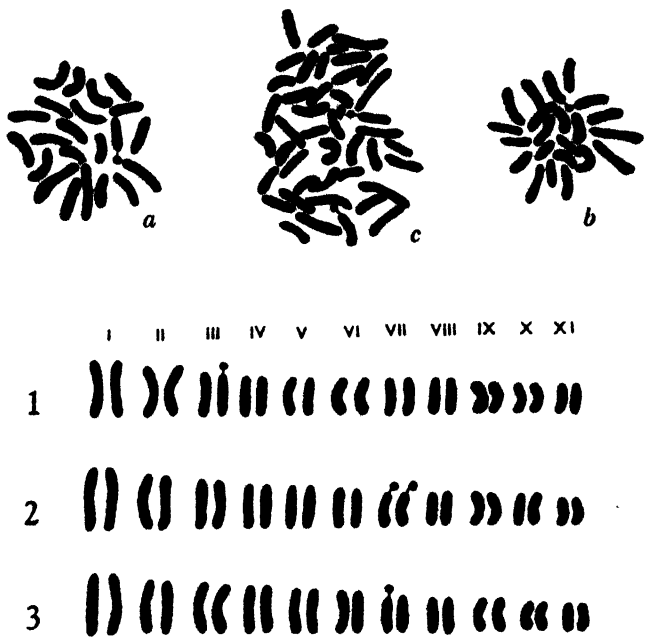

Fig. 1. *a*, *b*, somatic metaphases in *S. Němecii*. *c*, a tetraploid somatic metaphase in a tapetum cell in *S. Němecii*. Somatic idiograms: (1) *S. nutans*, (2) *S. Juriščii*, (3) *S. Němecii*. ×2400. (Drawn with Abbé's camera, obj. Zeiss apo. 120, ocul. Zeiss K 20.)

cases were discovered, either of two chromosomes with unequal satellites, or of only one chromosome with a satellite. The following references are only to the more interesting cases, and the list is not complete. M. S. Nawashin (1916, 1926) found similar cases in *Leontodon autumnalis* and *Crepis dioscoridis*, Němec (1935) in *Lilium candidum*. Philp & Huskins (1931) believed that in different ever-sporting races of *Matthiola incana* there was only one sat.-chromosome; but Westergård (1936) and Kuhn (1938) later showed that all races of *Matthiola* have two satellites, but that these were so small that previous investigators had overlooked the second one. But this is the only case in which a statement of nuclear asymmetry concerning the presence of satellites has been proved false. The other cases have been re-examined several times, or the size of the satellite is so great (e.g. in *Lilium* where one large rod-shaped satellite is present) that an error is quite impossible.

Such nuclear asymmetric plants are heterozygous, and their existence presupposes the inevitable existence of homozygous individuals or races, i.e. races with two large satellites

and those with two small ones, or alternatively, races with two satellites and races with none. Such races have actually been found in some cases. They were studied especially in *Crepis dioscoridis* from the genetical point of view by Medwedewa (1929). Benoist (1937) reported 22 chromosomes without satellites in *Salvia nutans*, but does not exclude the possibility of having overlooked them owing to the small number of mitoses investigated. I believe that nothing was overlooked in this case, and that Benoist had found the homozygous race without satellites. Hrubý (1934, p. 323) found a similar case in another species of *Salvia*, namely, *S. pratensis* L. Individuals with 18 chromosomes quite devoid of satellites were observed, whereas in other plants a pair of sat.-chromosomes was found. The existence of races quite without satellites (such individuals were found in the progenies of *S. Němecii* too) is also very interesting theoretically. There is to-day no doubt about the close relation between sat.-chromosomes and nucleoli, as formulated several times by Heitz (1931) and many other investigators. But the existence of individuals without satellites justifies Fernandes's (1936) opinion that while the sat.-chromosomes are always nucleolar chromosomes, nucleolar chromosomes may be wholly devoid of satellites. Thus the nucleolar organizer need not be localized in or near the satellite.

Only one sat.-chromosome has been found in the first spontaneous plant of *S. Němecii* and in all the hybrids artificially obtained by reciprocal crossing of the parent species. It is noteworthy that the sat.-chromosome is always derived from *S. Jurišićii*, as indicated by its length. In *S. nutans* the sat.-chromosome is one member of the third pair, whereas in *S. Němecii* it is shorter, and belongs to the seventh or eighth pair. It is thus a chromosome derived from *S. Jurišićii* in which the seventh pair consists of sat.-chromosomes. Theoretically we should expect half the plants of *S. Němecii* to have two satellites, but no such plant has been found in our cultures. As already stated (Hrubý, 1933) one F_2 plant was definitely shown to have a single satellite. The other two presumably had at least one, since plants with two, one, or no satellites were grown from their seed. Apart from changes in the chromosome number, caused by the occasional formation of univalents in meiosis, these three kinds should occur in the ratios 1 : 2 : 1. Actually two satellites occurred in 44 %, one in 16 %, and none in 40 %, that is, in the ratios 1·32 : 0·48 : 1·20. The deviation from the expected proportion may be due to the relatively small number of plants, but is more probably caused by changes in the chromosome number originating at meiosis as stated above. Two-thirds of the F_3 plants investigated had 22 chromosomes, the remainder only 20. In view of the occurrence of univalents at meiosis and the consequent origin of pollen grains with unequal chromosome contents, other numbers are also to be expected. Actually one such, namely 18, was found, as described in the final part of this paper.

MEIOSIS

In all species of the genus *Salvia* degeneration of the male organs is relatively frequent. In some individuals this process goes so far that only rudimentary staminodia are formed in place of fertile stamens, and such a plant becomes entirely female. At other times a similar process occurs in a normal plant, but in the later part of the flowering period or in the youngest parts of the inflorescence. These processes are much more striking in interspecific hybrids, in which the stamens in quite normal-looking flowers are often strongly reduced, or lack fertile pollen, though otherwise well enough developed. It follows that even in very young flower buds, those just convenient for the study of meiosis, we meet all possible stages of more or less advanced degeneration of the androecium. We find stamens

in which the archesporium already shows a necrotic character, like the tapetum and surrounding cells. In other flowers the degenerative process begins at the earliest stages of meiosis, for instance, at leptotene or later.

It is generally known that these early meiotic stages are particularly sensitive. Obviously in anthers where the necrosis of the archesporium is advanced or has even begun, we can find plenty of abnormalities whose description could fill several pages. All these forms are of course unimportant, being pathological conditions, and the cells containing them generally perish very quickly. The first trace of this process seems to be a loss of turgor in single cells and in the whole tissue. As a consequence not only cells but also their nuclei assume an amoeboid shape. Other anomalous phenomena, which are essentially artefacts, may often occur, especially when a strong fixative such as Carnoy is used, as the changes in osmotic pressure caused by penetration of the fixative may break the cell wall and further cause extrusion of a part of the deformed amoeboid nucleus from one cell into another. It stands to reason that even a very slight pressure during the manipulation of flower buds before fixing them may produce similar phenomena in quite normal and healthy anthers. In these ways the condition described as cytomyxis may originate.

This process, which many cytologists consider as an artefact in general, has been described in two other *Salvia* hybrids, by Scheel (1931) in *S. silvestris* L. and by Glišić (1934) in a spontaneous hybrid between *S. Jurišićii* Koš. and another species. To judge from Glišić's account of the chromosome number in his hybrid, the second parent may be a hybrid too, namely, *S. silvestris* L. This would explain the complete sterility of that hybrid, as well as many anomalous phenomena at meiosis which Glišić thoroughly describes and discusses. But the evidential value of his observations, as I have already pointed out (Hrubý, 1935, 1941), is much weakened, since he used haematoxylin stain. I have also found figures suggesting cytomyxis in my slides, but it has been shown that this appearance originated either by optical projection of cells and of deformed amoeboid nuclei, or through cutting the objects. Hereby a very thin part only of one cell layer is cut off, lying over or under the cell layer observed. In these cases the true situation may be shown by moving the micrometer screw very carefully. In spite of all this, I do not wish to deny the existence of cytomyxis completely, even though I have never seen it in several *Salvia* interspecific hybrids. It may occur in dying cells. The question could perhaps be solved in smear preparations stained with acetocarmine or Feulgen, where the danger of an error due to cutting would be eliminated. Tischler (1943, pp. 330, 530) has already given a thorough survey of the literature concerning cytomyxis.

In any case all these abnormalities in degenerating pollen mother cells, while interesting from the point of view of cellular pathology, are of no significance for the further course of meiosis, since the cells in question perish before meiosis could be completed. As our interest in meiosis concerns the origin of gametes capable of fertilization, we carried out our observations of it only on preparations where all anthers were quite normal, without any trace of even the beginning of degeneration. Anthers may sometimes degenerate much later, when the pollen has already been produced, but this had no influence on meiosis. Such pollen grains finally perish, together with the surrounding tissue. The following course of meiosis has been observed in quite normal anthers.

Attention was first paid to diakinesis and the first metaphase. In most cases eleven conjugated bivalents can be seen near the nuclear membrane at diakinesis (Fig. 2, 1). Idiograms of somatic chromosomes in both parent species show a remarkable morphological

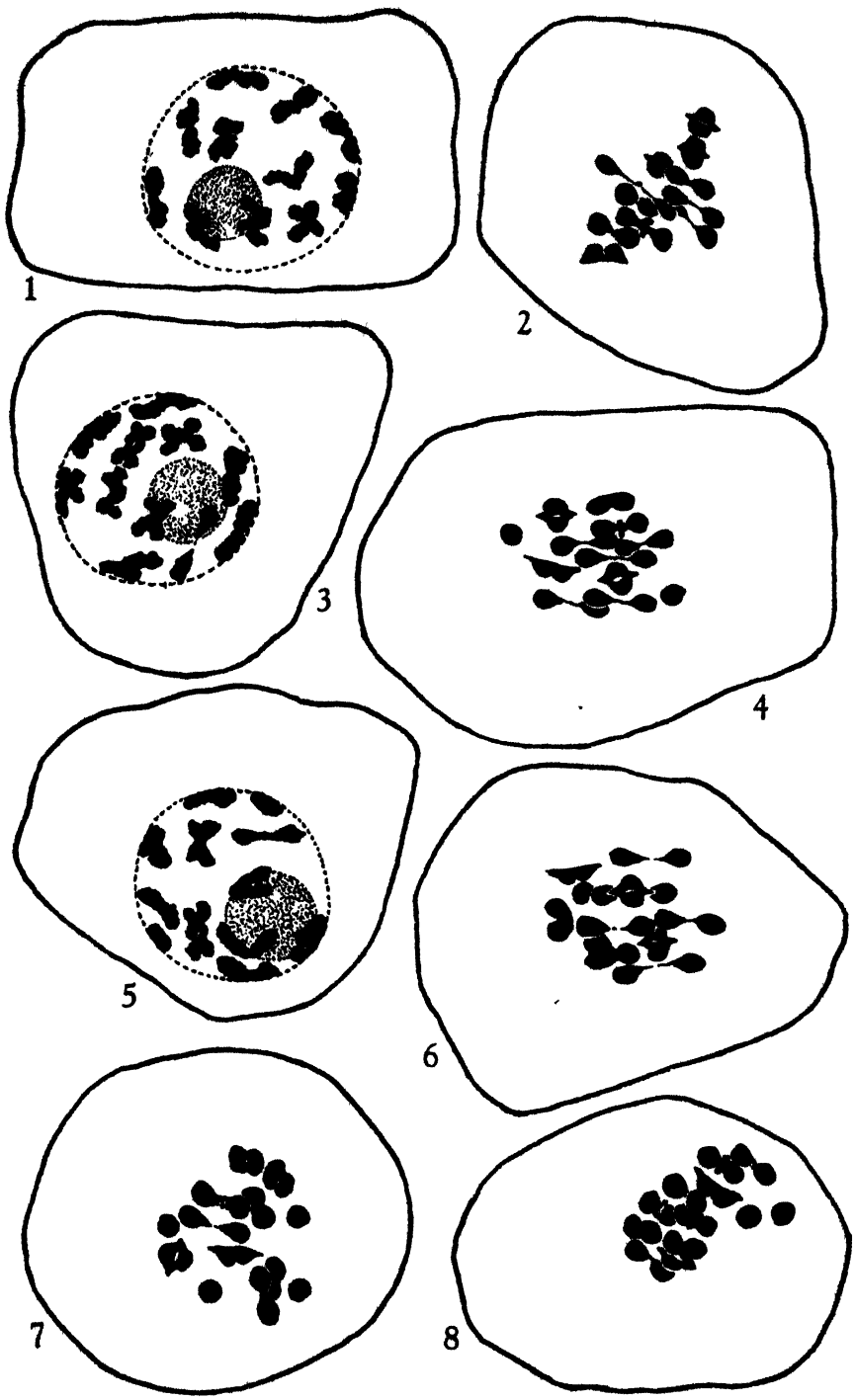

Fig. 2. Meiosis in *S. Němecii*; first division. (1) Diakinesis with 11 bivalents. (2) Metaphase with 11 bivalents. (3) Diakinesis with 11 bivalents, one of them not completely conjugated. (4) Metaphase with 10 bivalents and 2 univalents in typical position. (5) Diakinesis with 10 bivalents and 2 univalents. (6) Metaphase with 1 trivalent, 9 bivalents and 1 univalent. (7) Metaphase with 9 bivalents and 4 univalents. (8) Metaphase with 10 bivalents and 2 univalents, situated towards the same pole. × 2400.

similarity. There must also be a considerable physiological and genic affinity between them, otherwise complete pairing would not be so frequent. Nevertheless, the affinity between the two chromosomes seems to be somewhat reduced, as they are sometimes not completely conjugated (Fig. 2, 3), and in a certain number of cases they occur as univalents (Fig. 2, 5). No other abnormalities were found at diakinesis. At this stage and at the first metaphase chiasma formation may be observed. But the very small size of the chromosomes, and their relative shortness at meiosis, make an exact determination of chiasma frequency and localization very difficult. It is probable that chiasma frequency is low, and that a considerable terminalization takes place.

In accordance with the situation found at diakinesis, we usually find 11 chromosomes at the first metaphase also, the shapes and sizes of which are not too different (Fig. 2, 2). Univalents, most frequently 2, have also been found, occupying typical positions on the spindle out of the equatorial plane, one on each side of it (Fig. 2, 4). In other nuclei one univalent lies among the conjugated chromosomes and the second beside them on the spindle, or both univalents are out of the equatorial plane, but on the same side of it towards one pole (Fig. 2, 8). Other configurations were rarely found; for example, 9 bivalents, 1 univalent and 1 Y-shaped trivalent (Fig. 2, 6) and 9 bivalents disorientated in the cell and 4 univalents (Fig. 2, 7). In one case only I found 1 quadrivalent, 8 bivalents and 2 univalents, but the nature of the quadrivalent-like formation is not quite clear. Most probably there are two bivalents agglutinated or lying closely side by side, without the formation of chiasmata. The frequency of different configurations is given in Table 1, which is based only on those nuclei where the situation was quite obvious.

Table 1. *Frequencies of configurations at first meiotic metaphase in* Salvia Němecii

Configuration	Frequency %
11^{II}	62·0
$10^{II}, 2^{I}$	36·0
$9^{II}, 4^{I}$	0·5
$1^{III}, 9^{II}, 1^{I}$	1·0
$?1^{IV}, 8^{II}, 2^{I}$	0·5

The anaphase is quite normal when 11 bivalents are present, and in most other cases 11 chromosomes reach each pole. But sometimes both univalents move to the same pole, or one of them remains between the interkinetic nuclei. Its subsequent fate is described later. The interkinetic nuclei are slightly flattened, and their chromosomes do not disappear completely, but remain as distinct chromocentres near the nuclear membrane as in diakinesis. Hence they can be counted during interphase. Formation of chromocentres in resting nuclei seems to be common in *Salvia*. Benoist (1937) has found it in several members of the section *Plethiosphace*.

There are generally less abnormalities at the second division, so in our hybrid in which even the first division is fairly regular, we generally find regular divisions with 11 globular or ellipsoid chromosomes at the metaphase (Fig. 3, 2). Their shapes correspond approximately to those seen in the diploid phase. In 78% of all cases investigated, 11 chromosomes were found at the second metaphase, in 13% only 10 were found, whilst in 9% 12 were found. This variation is explicable by the irregular distribution of univalents in the first division. Thus two nuclei side by side in the same pollen mother cell (Fig. 3, 1) had 10 and 12 chromosomes, and both univalents had moved to the same pole at the first anaphase. But half the cases of second metaphases with unequal numbers arose in another way. One

univalent remained in the cytoplasm of the pollen mother cell between the interkinetic nuclei. The second reached a pole together with other chromosomes and took part in forming a normal nucleus. Thus in the second division one metaphase contains 11 chromosomes as usual, the other only 10, while the isolated univalent can be seen between the two metaphases, either as a single body (Fig. 3, 3) or just dividing (Fig. 3, 4). These isolated chromosomes are either resorbed later in the cytoplasm, or form very small nuclei, karyomeres, and tiny pollen grains. But nearly all pollen grains are quite normal, and there are no striking differences in the sizes of mature pollen, as their eventual chromosome contents differ little.

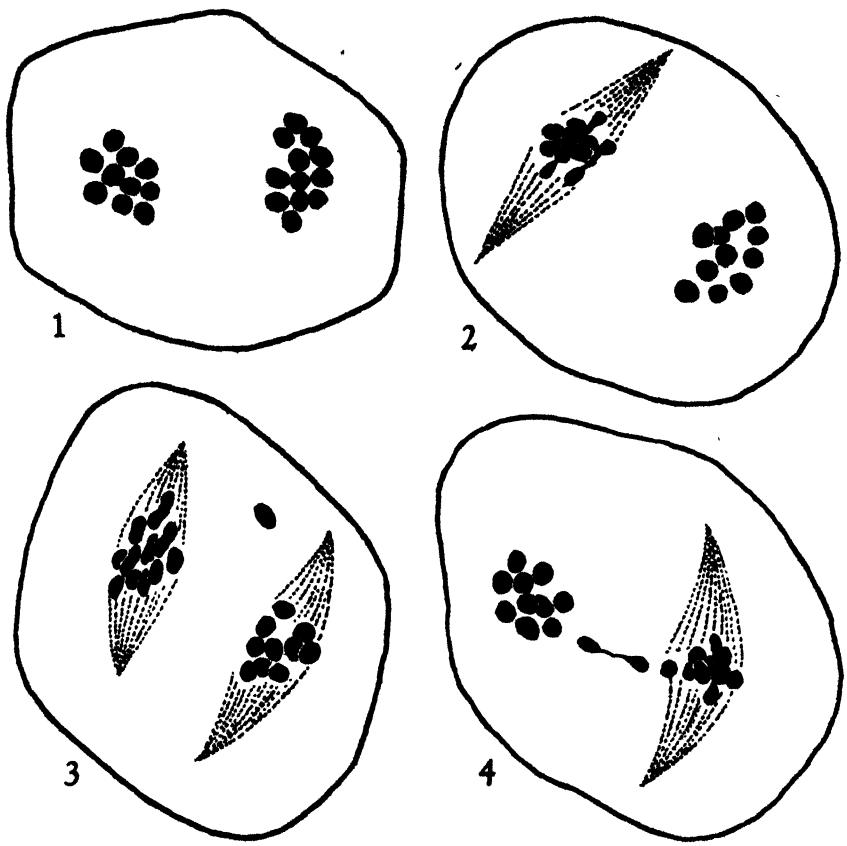

Fig. 3. Meiosis in *S. Němecii*; second division. (1) Two metaphases, one with 10, the second with 12 chromosomes. (2) Normal metaphases containing 11 chromosomes. (3) One metaphase with 10 chromosomes, the other with 11, 1 univalent between them in the cytoplasm. (4) A similar case to the preceding, but the univalent is just dividing. × 2400.

In F_2 plants univalents seem to be formed much more frequently at the first division, and consequently unequal numbers are commoner at the second metaphase. The make-up of the F_3 plants accords with this fact. In 66 % of them 22 chromosomes were found in somatic cells of the root-tip meristem, in 34 % only 20 chromosomes.

Darlington (1937, pp. 168 et seq.) distinguishes three types of structural hybrid according to the pairing at meiosis. 'Undefined structural hybrids are those whose parental gametes either had the same number of chromosomes or a different number, through a change that cannot be identified. They may be said to be undefined simply because the structural differences between their chromosomes are too slight or too numerous to be readily

detected' (Darlington, 1937, p. 137). To the first type belong those with complete or almost complete pairing at the metaphase; the second is formed by those with partial, and always variable, pairing; the third contains those hybrids with little or no pairing. The facts given above justify the assignment of our hybrid *S. Němecii* to the first class, of which also most *Salvia* hybrids so far cytologically investigated are members.

The dwarf plant

Among the F_3 plants cultivated before the war in Brožek's Genetic Garden (experimental garden of the Plant Physiology Laboratory of the Charles University, Prague), one plant of very striking general appearance occurred. This was a seedling of the F_2 plant denoted by A in the preliminary report (Hrubý, 1933), which in general resembled the parental species *S. nutans*. This plant had the habit characteristic of the growth mutations, usually called dwarf mutations, which arise especially after X-ray treatment. When fully developed it was only 5 in. (13 cm.) high, dense, bushy, with small, agglomerated but not too rugose leaves. The inflorescences were straight, formed of dense verticillasters of very small

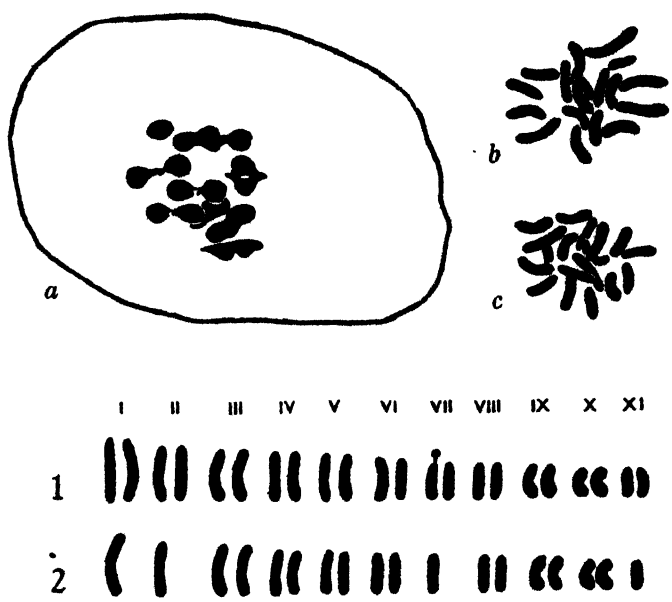

Fig. 4. The dwarf plant. *a*, first metaphase in ovule with 1 trivalent, 7 bivalents and 1 univalent. *b*, *c*, somatic metaphases with 18 chromosomes. Somatic idiograms: (1) normal *S. Němecii*, (2) the dwarf plant. × 2400.

flowers. It proved to be completely sterile. Various pollinations failed, as did vegetative reproduction, which was tried very carefully, in view of the general weakness of the plant. Though it was given all possible care, it died in 1936; but many flower buds were fixed, and preparations investigated in 1945.

Such a peculiar plant might have originated in three possible ways. The first is a true gene mutation or several simultaneous mutations. The second is the Mendelian segregation of growth factors, as obtained and described by Appl (1928) in the specific hybrid *Origanum majorana* × *O. vulgaris*. The third is a chromosomal mutation, i.e. a considerable change in chromosome number causing a quite new genotypic constitution of the individual. This last possibility proves to be the most likely, though the co-operation of either of the former cannot be entirely excluded, as, owing to the complete sterility of the plant, its genetical analysis was impossible.

The cytological examination was rather difficult. The sterility of this plant was due, not only to the complete failure of development of the stamens, which generally did not even form the archesporium, or shrivelled just after its formation, but also to a very advanced degeneration of most ovules. It was thus hard to determine the somatic chromosome number in ovular integuments. In some flower buds not a single metaphase was found. But a considerable number of slides made possible a reliable determination of the chromosome number, which is 18. Two metaphases are shown in Fig. 4*b*, *c*.

On comparing the idiogram of the dwarf plant with that of the normal *S. Němecii* (Fig. 4, 1, 2) we see that it lacks one chromosome from each of four pairs, namely, the two largest, I and II, the sat.-chromosome of pair VII, and one of the smallest pair XI. The loss of 4 chromosomes may have been due to the fusion of gametes which arose by meiosis in cells containing 4 univalents. It will be remembered that meioses of the postulated type have been found in *S. Němecii*. Another possible origin of gametes of lower chromosome content is non-disjunction. Meiosis was found in one ovule only. At the first metaphase 7 bivalents, 1 trivalent and 1 univalent were found (Fig. 4*a*). The trivalent probably arose by conjugation of the single chromosome of the second pair with the bivalent formed by the third pair. The single chromosomes of the seventh and eleventh pairs seem to conjugate end to end, whilst the one chromosome of the first pair remains as an univalent. More detailed investigation of this plant is unfortunately impossible. We may hope that other interesting individuals may be found among new cultures of *S. Němecii*.

SUMMARY

Salvia Němecii Hrubý is an interspecific hybrid between *S. nutans* L. and *S. Jurišićii* Koš. It occurred spontaneously among cultivated plants for the first time, and later it was obtained by means of artificial pollination. The author succeeded in both reciprocal crosses. All these plants were identical.

The somatic chromosome number of this hybrid is 22; one chromosome is provided with a globular satellite. A comparison of idiograms of both parent species and the hybrid is given. The question of nuclear asymmetry, viz. the occurrence of only one sat.-chromosome in *S. nutans* and *S. Němecii* is discussed. There are plants in F_3 having either two satellites, only one satellite or no satellite at all. The chromosome number 22 occurs in 66 % of F_3 plants, but the other individuals have only 20 chromosomes.

One completely sterile dwarf plant has been found among F_3 plants, having 18 chromosomes.

Meiosis in *S. Němecii* (F_1) was studied, and the origin of abnormalities in *Salvia* species hybrids is discussed. In normal flower buds, the course of meiosis is regular enough in *S. Němecii*. In most cases there are 11 bivalents. In 36 % 10 bivalents and 2 univalents were found, the behaviour of univalents being typical. Other cases are very scarce. Most gametes contain 11 chromosomes, few only different numbers, viz. 10 or 12 chromosomes.

Salvia Němecii belongs to the class of undefined hybrids characterized by potentially complete pairing.

The author is much indebted to the Czech National Research Council for support that assisted him in carrying on these investigations in the very worst years of the war.

REFERENCES

APPL, J. (1928). Über einen Bastard von *Origanum majorana* und *Origanum vulgare* und dessen Aufspaltung in der F_2 Generation. *Preslia*, **6**, 1–13.

BENOIST, E. (1937). Recherches caryologiques sur quelques espèces du genre *Salvia*. *Rev. Cytol. Cytophysiol. végét.* **2**, 415–40.

DARLINGTON, C. D. (1937). *Recent Advances in Cytology*, 2nd ed. London.

FERNANDES, A. (1936). Les satellites chez les Narcisses. II. Les satellites pendant la mitose. *Bol. Soc. Broteriana* (2nd sér.), **11**, 87–146.

GATES, R. R. (1939). A new method of staining. *Chron. Bot.* **5**, 191.

GLIŠIĆ, L. M. (1934). Cytological observation on a *Salvia* hybrid. *Bull. Inst. bot. Univ. Belgrade*, **3**, 1–17.

HEITZ, E. (1931). Die Ursache der gesetzmäszigen Zahl, Lage, Form und Grösze pflanzlicher Nukleolen. *Planta*, **12**, 775–844.

HRUBÝ, K. (1933). Preliminary report on *Salvia nutans* L., *S. Jurišićii* Košš. and the probable hybrid thereof. *J. Genet.* **27**, 471–82.

HRUBÝ, K. (1934). Zytologie und Anatomie der mitteleuropäischen Salbei Arten. *Beih. Bot. Zbl.* **52**/A 298–380.

HRUBÝ, K. (1935). Some new *Salvia* species hybrids, their description and analysis. *Stud. Pl. Physiol. Lab. Charles Univ.* **5**, 1–73.

HRUBÝ, K. (1937). Výroba trvalých mikroskopických preparátů botanických . [Making of permanent slides from Botanical objects—Czech.] *Věda Přír.* **18**, 182–4, 209–12, 245–50.

HRUBÝ, K. (1941). Untersuchung von zwei weiteren *Salvia* Artbastarden. *Věstn. Král. Čes. Spol. Nauk.* (*Proc. Boh. Roy. Soc.*), pp. 1–13.

KUHN, E. (1938). Über die Trabantenchromosomen bei normalen und immerspaltenden Sippen von *Matthiola incana*. *Z. indukt. Abstamm.- u. Vererb.-lehre* **74**, 388–408.

MEDWEDEWA, B. G. (1929). Über die Trabanten bei *Crepis dioscoridis* L. *Z. Zellforsch.* **10**, 150–63.

NAWASHIN, M. S. (1916). Случай несоразмѣрности ядер у Сложноцвѣтных. [A case of nuclear asymmetry in *Compositae*—Russ.] Журн. Русск. бот. общ. (*J. of Russ. Bot. Soc.*), **2**, 3–4.

NAWASHIN, M. S. (1926). Variabilität des Zellkerns bei *Crepis* Arten inbezug auf die Artbildung. *Z. Zellforsch.* **4**, 171–215.

NAWASHIN, S. G. (1912). О диморфизме ядер в соматических клетках у *Galtonia candicans*. [Nuclear dimorphism in somatic cells in *Galtonia candicans*—Russ.] Изв. Ак. Наук (*Proc. Acad. Sci.*), **6**, 373–85.

NAWASHIN, S. G. (1927). Zellkerndimorphismus bei *Galtonia candicans* Des. und einigen verwandten Monokotylen. *Ber. dtsch. bot. Ges.* **45**, 415–28.

NĚMEC, B. (1935). Über Frucht und Samenansatz bei *Lilium candidum* L. *Věstn. Král. Čes. Spol. Nauk.* (*Proc. Boh. Roy. Soc.*), pp. 1–16.

PHILP, J. & HUSKINS, C. L. (1931). The cytology of *Matthiola incana* R.Br., especially in relation to the inheritance of double flowers. *J. Genet.* **24**, 359–404.

SCHEEL, M. (1931). Karyologische Untersuchung der Gattung *Salvia*. *Bot. Arch.* **32**, 148–208.

TISCHLER, G. (1943). *Allgemeine Pflanzenkaryologie*. I. *Der Ruhekern*. Berlin 1934. II. *Kernteilung und Kernverschmelzung*. Berlin.

WESTERGÅRD, M. (1936). On the satellites in the ever-sporting *Matthiola* races. *C.R. Trav. Lab. Carlsberg* (Sér. Physiol.), **21**, 195–204.

GENETIC STUDIES IN POULTRY

X. CREAM PLUMAGE

By R. C. PUNNETT, F.R.S.

(With Plates 12–14)

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Introductory

Thirteen years ago I recorded some experiments dealing with blue egg colour derived from certain hens imported from Chili (Punnett, 1933). In the course of these experiments an unknown type of plumage colour made its appearance, and it is with this that the following brief account deals. In tracing the heredity of egg colour a Chilean hen of a nondescript light yellowish brown appearance was mated with a Gold-Pencilled Hamburgh cock. The F_1 birds were all gold with irregular pencilling. In a small F_2 generation bred subsequently there appeared some birds with a very pale creamy ground colour in addition to those of the normal gold type. The proportion in which these creams appeared pointed obviously to their being simple recessives to gold, and this was confirmed by the fact that they bred true in respect of the cream ground. The small stock of creams so obtained, though uniform in respect of ground colour, was highly diverse in the development and distribution of melanic pigment. With a view to further study it was decided to produce 'cream' forms of certain well-established types of plumage, and for this purpose the Buff and the Brown Leghorns were chosen. And here mention must be made of L. W. Taylor's work (1932) with birds which he also described as creams. He found the character to be recessive to gold, and I feel little doubt that we were simultaneously working with similar material. Our interpretations, however, are somewhat different; for while he regards the 'cream' gene as an inhibitor of gold I prefer to look upon gold as being due to a gene which intensifies cream. More recently Mr M. S. Pease has informed me that cream has turned up in one of his crosses, and it is not unlikely that the character is more widely spread than is generally realized.

The Buff Leghorn cross

A Buff Leghorn ♂ was mated with 2 cream ♀♀ in 1934 and gave only light gold offspring. An F_2 generation from 2 ♂♂ and 7 ♀♀ produced in the following years resulted in 113 golds and 45 cream. F_2 birds with the minimum of melanic pigment were selected to produce an F_3 generation in 1936. With one exception the chicks were all 'creamy' in down and developed into adults with cream plumage and very little melanic pigment. It was noted

that some of the ♀♀, particularly those with more melanic pigment, showed a slight gold tinge, whereas in the ♂♂ not only was this gold tinge never present, but the cream tinge was so faint that they could easily be mistaken for silvers (cf. Pl. 12, fig. 1). Subsequent experience confirmed this sexual difference. Reduction of melanic pigment, such as occurs in the 'Columbian' pattern, brings about a rich cream ground colour in the hen, whereas the corresponding cock might be said to mimic a silver, though a faint straw tinge in the hackles tends to become accentuated as the feathers age. I have bred many creams with the Columbian pattern, but I have never seen a cock with the cream ground colour characteristic of the hen, nor have I ever had a hen which, like the cock, could be mistaken for a silver. It would be interesting to carry out castration experiments with such a strain.

The Brown Leghorn cross

The object of this cross was to ascertain the appearance of the birds when cream was substituted for gold in the Brown Leghorn type of plumage. A Brown Leghorn ♂ was mated with 2 F_2 cream ♀♀ from the cream × Buff Leghorn cross, which of course had a type of plumage approaching the Columbian. In down the chicks showed a good deal of variation. Some were of a normal brown stripe; others were paler, sometimes with the stripe well marked, and others with the stripe so blurred that the down was of a more or less uniform golden brown. Such variation must, I think, be due to unanalysed differences in the make-up of the Columbian cream strain in spite of the fact that they showed little difference among themselves phenotypically. So far as was noted this variation was not connected with the sex of the chicks, but I do not wish to stress this point since little heed was paid to it at the time. The birds reared all grew up into golds with nondescript melanic markings which were more pronounced in the hens than in the cocks. Of these F_1 birds 2 ♂♂ and 10 ♀♀ were mated up in a single pen with the object of obtaining the cream black-red as quickly as possible. Of the 225 birds which survived long enough for colour determination 172 were golds and 53 were creams. The golds were discarded as soon as they could be recognized with certainty. As in the F_1 generation, down colour showed great diversity, but although points of interest arise in connexion with them I prefer not to discuss them since I have been unable to come to any definite conclusions.

Of the cream F_2 birds reared to maturity the darkest of the pullets closely approached the general coloration of the Brown Leghorn ♀. These were all brown striped in the down. But of the few cream cockerels successfully reared from brown-striped downs not one approached the Brown Leghorn ♂ in plumage. They were of a predominantly light type, a good deal splashed with chestnut and black. The bird figured on Pl. 12, fig. 2 represents such a 'splashed' bird though in this case the splashing is more reduced than usual.

Failure to recover the Brown Leghorn type of plumage in the F_2 ♂ necessitated a further mating in the following year. For this purpose a 'splashed' F_2 ♂ was mated with 7 F_2 pullets all with plumage approaching that of the Brown Leghorn ♀. These pullets were all dark-shanked whereas the ♂ was a heterozygous light-shanked bird. The interest of this will appear later. From this mating all of the chicks hatched out brown striped. Ninety of these were reared to the adult or nearly adult stage, viz. 52 ♀♀ and 38 ♂♂. Both ♂♂ and ♀♀ could be divided with fair accuracy into two classes. For the ♀♀ the distinction was breast colour which was either full or nearly full salmon, or else was either pale salmon or only salmon tinged. For the ♂♂ the two classes were concerned with general plumage type,

whether splashed, as in the male parent, or approaching nearly to the Brown Leghorn type as shown on Pl. 12, fig. 3. In either sex the two classes were of approximately the same size and evidently depended on the transmission by the male parent of some inhibitory element occurring in the Columbian pattern for which he must have been heterozygous.

Here we may revert for a moment to the point raised above in connexion with shank colour. The male parent, as already noted, was heterozygous for both shank colour and the inhibitor of plumage pigmentation. In respect of these two pairs of characters his 38 sons may be classified as follows:

	Dark shanks	Light shanks
Light plumage	6	14
Dark plumage	13	5

Though the figures suggest a linkage between these two inhibitors the numbers are few, and I have given them with the idea that it may draw the attention of some future worker to this point.

The figures for the two classes of ♀♀, though still suggestive of linkage, are not so marked. On the supposition that the 'Columbian' inhibitor here inhibits the production of salmon on the breast the classified figures are:

	Dark shanks	Light shanks
Pale salmon	15	12
Full salmon	16	9

The excess of dark-shanked birds tends to mask the somewhat more frequent association of pale breast with light shanks.

However, the chief interest of the above mating is that not only pullets but cockerels also were produced which were very close to the Brown Leghorn type of plumage. In the following year two pens were mated up, each with a ♂ and 2 ♀♀ of birds nearest to the type desired. From these was ultimately established a strain with typical Brown Leghorn plumage, but on a cream ground instead of on a gold one. In such a strain the hen closely resembles a silver grey, though close inspection shows that the general tint is just a shade warmer, though less warm than in the Brown Leghorn, and that the neck hackles are straw tinged. In the cock, however, with his more abundant hackles, the distinction is far more obvious. For the white edging of these feathers (Pl. 14, fig. 4) in place of the normal gold (Pl. 14, fig. 3) brings about a very different appearance. Again, the chestnut of the wing covers is not so intense as in the Brown Leghorn (cf. Pl. 14, figs. 5 and 6, and figs. 7 and 8). Nevertheless, though less intense, chestnut in the Brown Leghorn remains chestnut in its cream counter-part. The difference in ground colour between cream and gold offers a chance of distinguishing in a black-red between coloration due to gold and that due to chestnut. Such a test is the outer web of the secondaries which in the Brown Leghorn is of a bright gold-brown. On Pl. 14, figs. 1 and 2 are shown two corresponding secondaries of a normal Brown Leghorn ♂ and a cream. The bright brown outer web of the former is replaced in the latter by white. The inference is that in the black-red ♂ this part of the colour scheme is dependent on the development of gold pigment, and is independent of chestnut.

THE RHODE ISLAND RED CROSS

Some experiments were made by crossing Rhode Island Red with cream—the idea behind them being to ascertain the extent to which the chestnut element in the Rhode Island Red make-up could be separated from the gold basis on which it was developed. The Rhode

Island Red was crossed with the pale cream derived from the Buff Leghorn cross. For various reasons the work was not carried far, but enough evidence was collected to show that the chestnut element in the Rhode Island Red could be shifted from its normal gold on to a cream basis. Hens were bred in which the chestnut was fairly evenly spread on a cream basis to give a cold form of chestnut. But so far as the work went no fully chestnut ♂ was bred. Even in birds with the most chestnut there was always a considerable admixture of cream feathers irregularly dispersed in the plumage.

Genetical relation of silver, gold and cream

Gold and cream form an allelomorphic pair, but, as is well known, gold and silver also form an allelomorphic pair which is extensively used for sex-linkage in commercial breeding. Either we must suppose that silver, gold and cream form an allelomorphic series located in the sex chromosome, or else we must consider that we are dealing with two independent pairs, one situated in the sex chromosome and the other in an autosome. And if the latter supposition turns out to be the more likely one, what is the fourth term required to constitute our two allelomorphic pairs?

We may consider first the hypothesis of a triple allelomorphic series situated in the sex chromosome. This hypothesis was put to an experimental test in the following way. By a happy chance a silver Light Sussex ♀ was found which on mating with a cream ♂ gave only silver ♂♂ and cream ♀♀. Such ♂♂ must be silver on cream, and one of them mated back to cream ♀♀ gave silvers and creams of both sexes. A silver hen from this mating (i.e. silver on cream) was mated with a gold Rhode Island Red ♂. If silver, gold and cream form an allelomorphic series in the sex chromosome the ♂♂ from this mating should receive silver from their mother and gold from their father, i.e. cream from neither parent. Hence such ♂♂ mated to cream ♀♀ should give silvers and golds of both sexes, *but no creams*. Actually this mating gave 13 silvers, 9 golds and 8 creams, both sexes being represented in each colour class. Clearly this disproves the hypothesis of an allelomorphic series in the sex chromosome. But the figures accord reasonably well with the assumption of two independent allelomorphic pairs where expectation would be silvers, golds and creams in the ratio 2 : 1 : 1.*

The question now remains as to what these two pairs are. Evidently silver is one member of a pair located in the sex chromosome. And in view of the fact that the Light Sussex hen already mentioned did not transmit gold when mated with a cream ♂, I incline to consider that the sex-linked pair should be regarded as a silver-cream pair. Hence, since the experimental evidence excludes the hypothesis of a multiple series in the sex chromosome, we must suppose that 'gold' is located in an autosome. If so, the most natural way of regarding it is to suppose that it is an intensifier of cream, transforming it to gold, and that it is allelomorphic to something which does not bring about the intensification of cream to gold. What that something may be we have at present no means of deciding. For the present we can only regard it as absence of the intensifier.

An alternative view is, of course, to suppose that gold and cream form an allelomorphic pair in some autosome, and that either may be inhibited by the 'silver' factor in the sex

* While this experiment was in progress I learned from Mr M. S. Pease that he had obtained the following evidence telling against the supposition of a multiple allelomorphic series. A gold hen carrying cream when mated with a cream cock gave golds and creams of both sexes. On the supposition of multiple allelomorphs one would have expected all the male chicks to be gold and the females to be cream.

chromosome. In that case we are left with the problem of deciding what the allelomorphic pair to the 'silver' inhibitor may be, and at present we can on this view regard it only as the absence of the inhibitor. A decision between these two views might be provided if we could establish a linkage between gold and cream and some other autosomal pair. That must remain for future work to decide. The only data that I can at present offer are negative, though for the benefit of future workers it may be worth while to set them out below.

SOME LINKAGE DATA

A mating was made between a ♂ which was single-combed (**r**), non-crested (**cr**) and proved not to carry the factor for blue egg (**b**), and 4 ♀♀, all of which were rose-combed (**R**), crested (**Cr**) and layers of blue eggs (**B**) in respect of which three characters they were heterozygous. At the same time all of the birds were heterozygous for cream (**Gg**). Symbolically the ♂ was $\frac{\mathbf{r\ cr\ b\ G}}{\mathbf{r\ cr\ b\ g}}$ and the ♀♀ were $\frac{\mathbf{r\ cr\ b\ G}}{\mathbf{R\ Cr\ B\ g}}$. The results for the various characters were as tabulated below:

Rose		Single			
Blue	Non-blue	Blue	Non-blue		(Coupling series)
13	13	10	13		
Rose		Single			
Crest	No crest	Crest	No crest		(Coupling series)
30	28	24	29		
Rose		Single			
Gold	Cream	Gold	Cream		(Repulsion series)
53	15	42	21		
Crest		No crest			
Blue	Non-blue	Blue	Non-blue		(Coupling series)
19	17	12	9		
Crest		No crest			
Gold	Cream	Gold	Cream		(Repulsion series)
36	18	41	16		
Blue		Non-blue			
Gold	Cream	Gold	Cream		(Repulsion series)
15	8	18	8		

Data have already been accumulated to show that three of the allelomorphic pairs here dealt with, viz. rose-single, crest-non-crested and blue-non-blue, are located in different autosomes (Hutt & Lamoreux, 1940). The data tabulated above, though scanty, offer no grounds for supposing that the gold-cream pair is located in any of these three autosomes.

A few further data are available in connexion with the rose-single pair. A gold RC ♂ heterozygous for cream of constitution $\frac{\mathbf{R\ g}}{\mathbf{r\ G}}$ was mated with two SC cream hens to give

Gold rose	9
Gold single	11
Cream rose	7
Cream single	6

At present the data given above are all that I can offer in connexion with the gold-cream pair, and although they show no obvious linkage with rose, crest or blue egg it should be borne in mind that these three characters are all located near the extreme end of their respective chromosomes. It is, therefore, not impossible that further data involving genes located elsewhere on one or other of these chromosomes may disclose a linkage for the gold-cream pair.

Summary

A type of plumage hitherto unrecorded in this country is described, viz. 'cream', which behaves as a simple recessive to gold. Silver, gold and cream do not constitute an allelomorphic series in the sex chromosome, but the three types of plumage depend upon two allelomorphic pairs of which one is located in the sex chromosome and the other in an autosome. The possible nature of these two pairs is discussed in the text.

Since 1942 part of the cost of these experiments has been met by grants from the Government Grants Board of the Royal Society, to which Board I wish to place on record my acknowledgement.

REFERENCES

Hutt, F. B. & Lamoreux, W. F. (1940). *J. Hered.* **31**, 231, 235.
Punnett, R. C. (1933). *J. Genet.* **27**, 465–70.
Taylor, L. W. (1932). *Proc. 5th Int. Congr. Genet. Ithaca.* 197–9.

EXPLANATION OF PLATES 12—14

Plate 12

Dorsal view of three male birds.

Fig. 1. 'Cream' cock with Columbian type of plumage. There is rather more melanic pigment in this bird than in the majority of those bred.

Fig. 2. 'Splashed' cream cock. Such birds generally show more chestnut and more melanic pigment than the one figured.

Fig. 3. Cream cock with Brown Leghorn type of plumage.

Plate 13

Ventral view of the three birds figured on Pl 12.

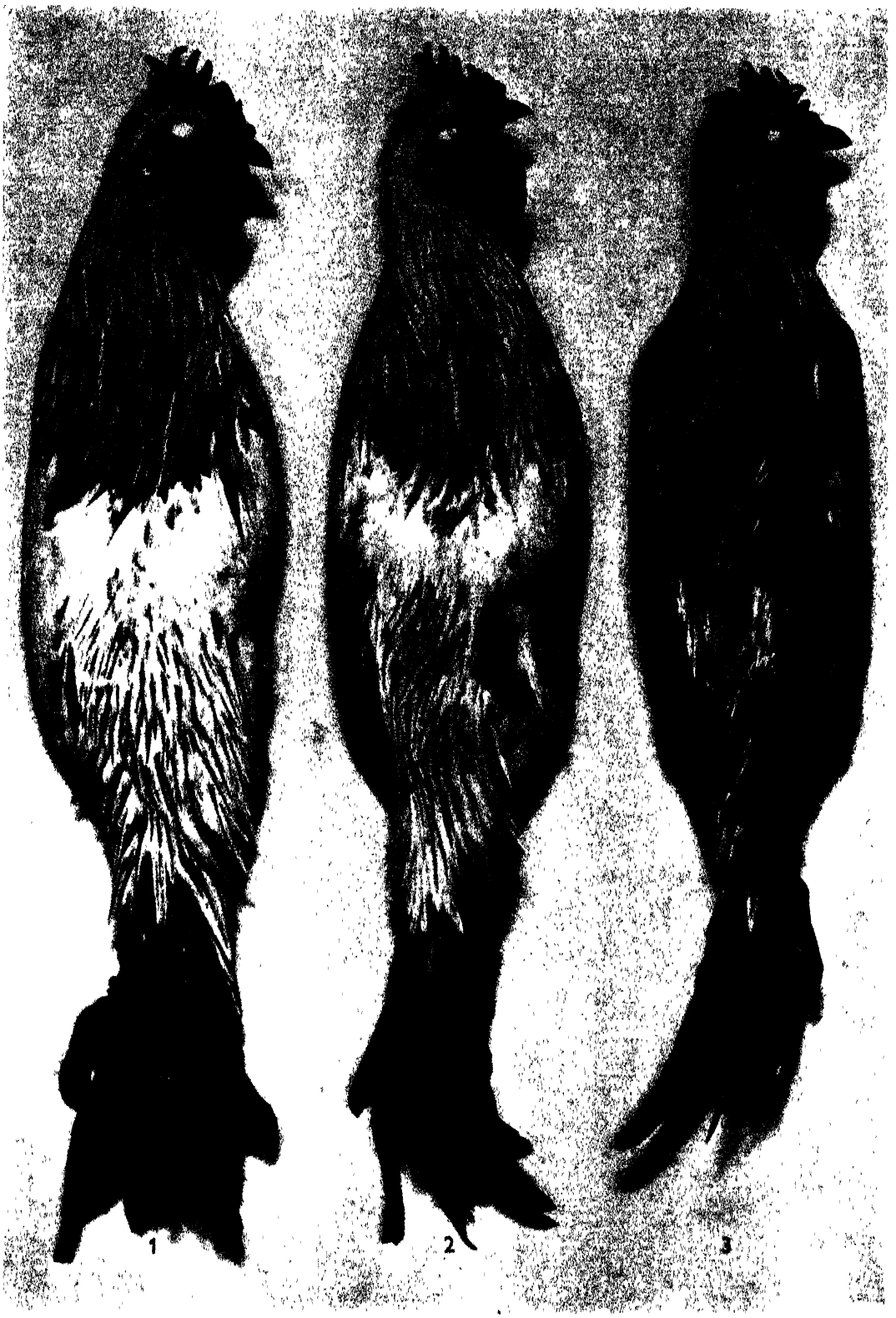
Plate 14

Corresponding feathers from a pure Brown Leghorn cock and from a cream with the Brown Leghorn type of plumage.

Figs. 1, 2. Secondaries.

Figs. 3, 4. Neck hackle.

Figs. 5–8. From wing coverts. Figs. 5 and 7 are from a cream and Figs. 6 and 8 from a Brown Leghorn.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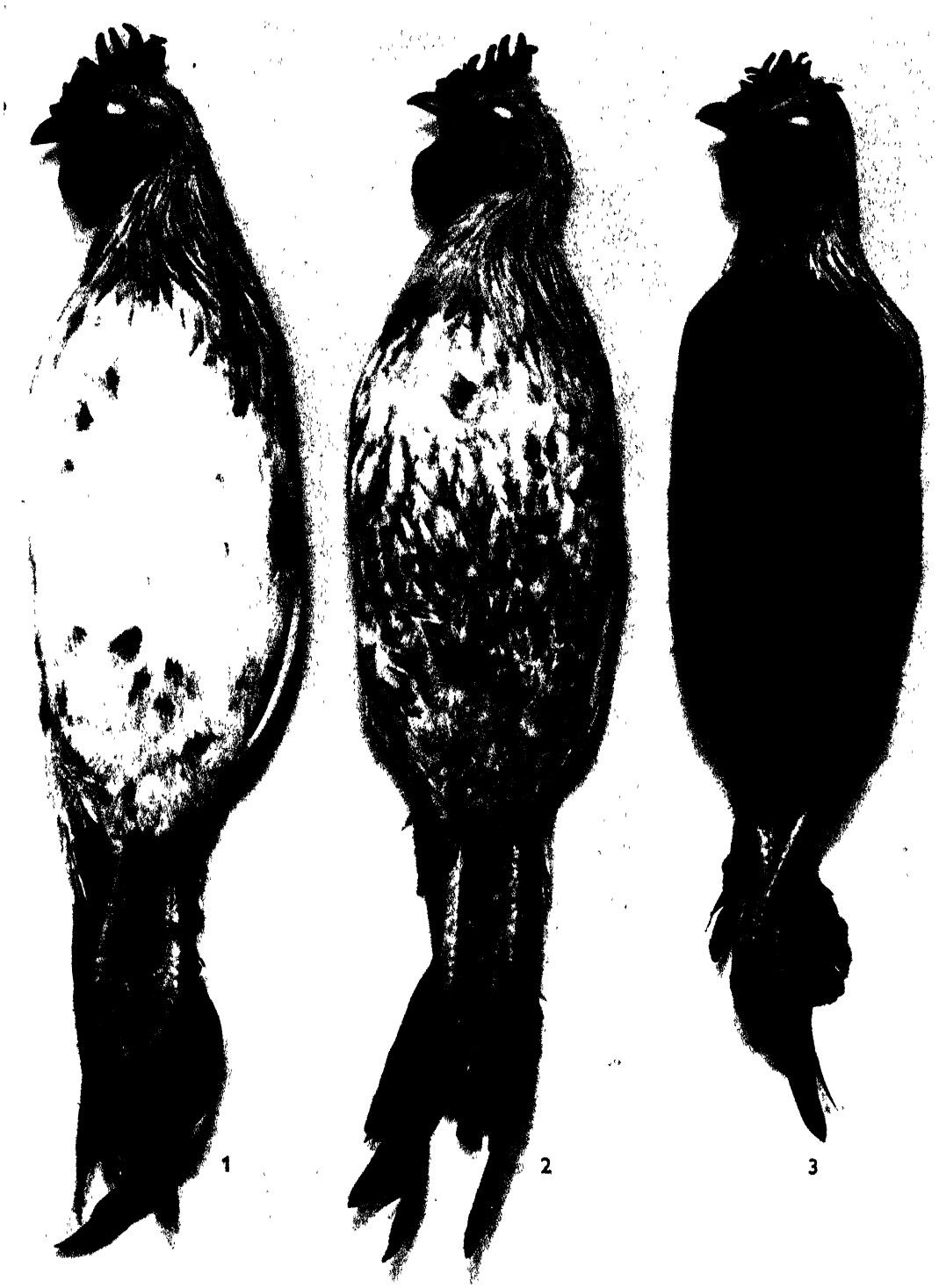
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THE SEX RATIO AND THE OCCURRENCE OF MUTATIONS IN CULTURES OF THE BLOW-FLY (*CALLIPHORA ERYTHROCEPHALA*)

By P. TATE, *From the Molteno Institute, University of Cambridge*

A white-eyed mutation of *Calliphora erythrocephala* was studied recently (Tate, 1947), and it was shown that the mutation is sex-linked and sex-limited, occurring only in the double recessive female. The mutant gene is located in the *X*-chromosome, and in the male there is a normal dominant allelomorph in the *Y*-chromosome which prevents the expression of the white-eyed character when the mutant gene is present in the *X*-chromosome of the male. During the many breeding experiments with pure-line cultures made for the genetical analysis many data were accumulated regarding the ratio of males to females in various strains, both mutant and wild-type, and a number of abnormalities occurred in the inbred cultures which may have represented gene mutations, although it was not possible to study them genetically.

Sex ratios in mutant and wild-type strains

In cultures containing the mutant gene it appeared that there was a deficiency of females and that the deficiency was most marked in pure-line white-eyed cultures. When all the flies bred from homozygous or heterozygous mutant strains were tabulated the figures obtained were 16,313 white-eyed females, 6148 heterozygous females and 26,700 males (Table 1). Thus there were 46 % females and 54 % males. The results for pure-line white-

Table 1. *The numbers and percentages of females and males bred from the various mutant lines, both pure-line white-eyed and heterozygous mutant strains of* Calliphora erythrocephala

Mutant culture no.	White-eyed females	Wild-type females	Wild-type males	% females	% males
1	589	262	943	47	53
2	861	861	1075	62	38
3	221	599	905	48	52
4	4465	72	5447	45	55
5	1182	1	1475	45	55
6	1495	12	1741	46	54
7	216	450	688	49	51
8	321	729	1095	49	51
9	54	1103	1399	45	55
10	642	349	1192	45	55
11	161	273	725	37	63
12	645	323	1174	46	54
13	17	119	199	41	59
14	45	281	410	44	56
15	2733	—	3691	43	57
16	2306	—	3357	41	59
17	154	497	734	47	53
18	206	217	450	48	52
	16313	6148	26700	46	54
	22461				

eyed cultures were tabulated (Table 2) and in eleven cultures there were 12,735 white-eyed females and 16,373 males, giving a sex ratio of 44 % females to 56 % males. Different

strains varied in the ratio of females to males, and the lowest was 40% females to 60% males and the highest 50% females to 50% males.

These results were compared with those obtained for three pure-line wild-type strains, each originating from a single wild-caught female. The combined figures for the three strains are: females 12,961 and males 14,919, giving a female to male ratio of 46% to 54% (Table 3). The different strains varied in the sex ratio, one having a female to male ratio of 50 : 50%; another having a ratio of 45 : 55% and the third a ratio of 47 : 53%.

There is not sufficient proof, therefore, for believing that in the mutant cultures females are more deficient than in cultures of wild-type strains, and it appears that in both wild-type and mutant strains of *C. erythrocephala* more males are produced than females under the conditions in which the breeding experiments were carried out. Much more detailed

Table 2. *The numbers and percentages of females and males bred from eleven pure-line white-eyed mutant strains of* Calliphora erythrocephala

Culture no.	White-eyed females	Wild-type males	% females	% males
1	450	454	50	50
2	270	267	50	50
3	120	177	40	60
4	974	1268	43	57
5	1127	1264	47	53
6	3834	4749	45	55
7	380	522	42	58
8	377	453	45	55
9	2733	3691	43	57
10	2306	3357	41	59
11	164	171	49	51
	12735	16373	44	56

Table 3. *The numbers and percentages of females and males bred from three pure-line wild-type strains of* Calliphora erythrocephala

Wild-type culture no.	Wild-type females	Wild-type males	% females	% males
1	547	539	50	50
2	5613	6794	45	55
3	6801	7586	47	53
	12961	14919	46	54

and extended work is required to settle the question, and it is possible that the results may be due, wholly or in part, to an unrecognized error in the breeding technique. In *Calliphora* the males develop more rapidly than the females, the first larvae to pupate are mostly males, and usually male flies emerge from the puparia 12–24 hr. before the females. Consequently, some female larvae tend to lag and relatively more of them may be discarded with unconsumed food than of males; and again, when counting the flies which have emerged each day it is necessary to anaesthetize them lightly with chloroform or ether. This may injure flies still within the puparia or those in the process of emergence and, as it is the females which are last to emerge, it may cause a slight differential mortality to the detriment of the females. This, however, would not explain the marked difference between different strains; and it is possible that further work will show that different strains, when maintained in pure-line culture, have inherent sex ratios differing from the 1 : 1 ratio to be expected in a mixed population, and that the tendency to produce more females than males becomes accentuated with successive generations of inbreeding.

POSSIBLE MUTATIONS OCCURRING IN INBRED CULTURES

Among the flies bred in studying the white-eyed mutant some other abnormalities were found, but it was not possible to investigate them genetically, because either the flies were already dead when the abnormal character was noticed, or they failed to give offspring, or the cultures died out before sufficient data had been obtained. The most interesting of these abnormalities are given in the following list.

(1) In a white-eyed culture one female was found which had a band of wild-type pigment extending across the left eye below the middle. The right eye was uniformly devoid of pigment. This abnormality might be due to somatic mutation or the individual may have been a gynandromorph although except for the eye the external morphology was of female type.

(2) In a white-eyed culture one male was found which had a small 'white' spot in the centre of each eye.

(3) In a white-eyed culture one female was found in which the left eye was wild-type whereas the right eye was white.

Attempts to breed from this interesting female failed. In *Drosophila* mosaics are usually attributed to the elimination of a chromosome at an early segmentation division. If an X-chromosome is eliminated from a female, gynandromorphs arise and male characters are exhibited by the part of the insect developing from cells lacking the second X-chromosome (Bridges, 1922; Waddington, 1939; Morgan, 1914; Sturtevant, 1929). The occurrence of the wild-type eye in the white-eyed female of *Calliphora erythrocephala* could not be explained by the elimination of an X-chromosome as the mutant gene is recessive. Careful examination of the abnormal female did not reveal any male characters, and the genitalia, both external and internal, were of the normal female type. Thus there is no evidence that this individual was a gynandromorph. The limited area of tissue affected makes it unlikely that non-disjunction could be the cause, and it is difficult to understand how a somatic mosaic showing a dominant character could arise in a double recessive female.

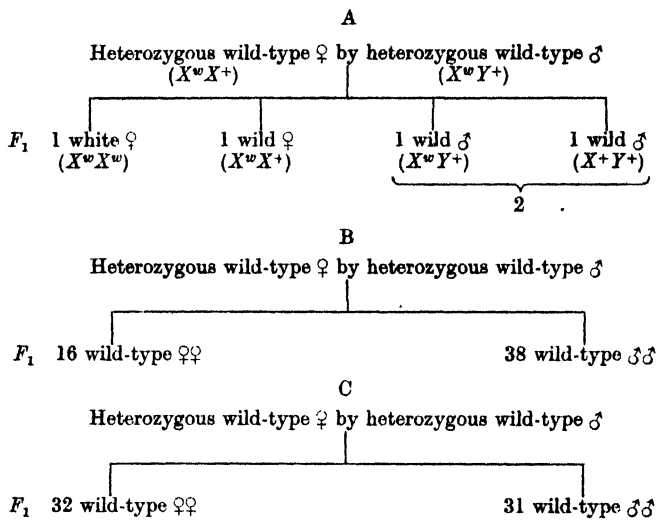
(4) In one wild-type strain derived from a single female which was maintained for twenty generations there was a high mortality in late larval life. Many fully grown larvae when ready for pupation emptied the gut and crop but then became rigidly extended and died without forming puparia. Other larvae became similarly extended and formed puparia without the customary contraction and retraction of the anterior segments of the body. The larvae curved dorsally as sclerotization developed, and abnormal puparia were formed which were slender, curved and tapered anteriorly, so that they were finally horn-shaped. The pupae invariably died within these monstrous puparia. The inheritance of this interesting abnormality was being investigated but the strain died out before crossing experiments with other strains were completed. Possibly some lethal gene was involved which acted in late larval and early pupal life.

(5) An interesting and puzzling deviation from the expected results of crossing heterozygous females with heterozygous males occurred twice. The F_1 progeny from such a cross should consist of white-eyed females, wild-type females (heterozygous), wild-type males (homozygous) and wild-type males bearing the white-eyed factor (heterozygous) in the ratios 1 : 1 : 1 : 1, the two types of males being indistinguishable morphologically (Table 4 A). In two cages containing such crosses, however, batches of eggs which gave only wild-type females were obtained. In one case there were 16 wild-type females and 38 wild-type

males (Table 4B); and in the second there were 32 wild-type females and 31 wild-type males (Table 4C). In the first case it is possible that the white-eyed females had died before emergence from the puparia as the number of females is only half that of the males, but in the second case the 32 females and 31 males were raised from 65 puparia and there is therefore no indication of a differential mortality among the pupae.

A possible explanation of these results would be the fertilization of the white-eyed female by a non-disjunctional male (XYY) producing X^wY and Y sperms. As the female was X^wX^+ the resulting zygotes would be X^wX^wY, wild-type female, X^+X^wY, wild-type female, and wild-type males of the constitution X^wY and X^+Y.

Table 4



A = expected progeny from cross-mating a heterozygous wild-type female with a heterozygous wild-type male. There should be equal numbers of white-eyed and wild-type females and all wild-type males, of which half would bear the mutant gene in the X-chromosome. B and C = results obtained from two batches of eggs obtained in breeding cages containing heterozygous wild-type females and heterozygous wild-type males.

X^w = X-chromosome with mutant gene. X^+ = X-chromosome with wild-type allelomorph. Y^+ = Y-chromosome with wild-type gene.

Summary

1. The sex ratio in pure-line cultures of strains of a white-eyed mutant of *Calliphora erythrocephala* varied from strain to strain. The average for 49,161 flies was 46% females and 54% males. Different strains varied in the ratio of females to males. The lowest was 40% females to 60% males and the highest 50% females to 50% males.

2. In cultures of wild-type *C. erythrocephala* it was found that on the average more males than females were produced. Of 27,880 flies, 46% were females and 54% males. Different strains gave different ratios of females to males, varying from 50% females to 50% males to 45% females to 55% males.

3. During extensive inbreeding experiments with pure-line cultures a number of possible mutations occurred, including some mosaics in which only part of the insect showed the mutant character.

4. In two cases when females heterozygous for the white-eyed mutant gene were crossed with males bearing the mutant gene in their X-chromosome, the progeny consisted of

wild-type males and only wild-type females, instead of white-eyed and wild-type females and males in the proportion 1:1:2. Possibly these cases were due to the fertilization of the parent females by males with non-disjunction of the XY-chromosomes.

REFERENCES

BRIDGES, C. B. (1922). The origin of variations in sexual and sex-limited characters. *Amer. Nat.* **56**, 51–64.

MORGAN, T. H. (1914). Mosaics and gynandromorphs in *Drosophila*. *Proc. Soc. Exp. Biol., N.Y.*, **11**, 171–2.

STURTEVANT, A. H. (1929). The claret mutant type of *Drosophila simulans*: a study of chromosome elimination and of cell-lineage. *Z. wiss. Zool.* **135**, 323–56.

TATE, P. (1947). A sex-linked and sex-limited white-eyed mutation of the blow-fly (*Calliphora erythrocephala*). *J. Genet.* **48**, 176–91.

WADDINGTON, C. H. (1939). *An Introduction to Modern Genetics*. London: George Allen and Unwin, Ltd.

THE STRUCTURE OF NORMAL AND MUTANT EYES IN THE BLOW-FLY (*CALLIPHORA ERYTHROCEPHALA*) AND THE DEVELOPMENT OF EYE PIGMENT DURING PUPATION

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(With One Text-figure)

During work on the genetics of a white-eyed mutant form of *Calliphora erythrocephala* it was necessary to compare the structure of the eye of this fly with that of *Drosophila*, especially as regards the number and arrangement of the pigment cells in normal and mutant eyes (Tate, 1947).

The structure of the eye of *Calliphora erythrocephala* has been described by Lowne (1893–5), but his description cannot be correlated with that described in recent work for other Diptera such as *Drosophila*. By studying the structure of the developing eye in pupae of various ages of both normal and mutant flies it has been found that in fact the eye structure is very similar to that of *Drosophila*, or, indeed, to that of *Vanessa urticae* as described by Eltringham (1919), especially as regards the arrangement of the pigment cells.

Methods

The best results in preparing eyes for sectioning were obtained by fixing the eyes for 2–3 hr. in Carnoy's fluid, embedding in paraffin wax of 52° C. melting-point, and cutting sections 7–10 μ thick. The sections were mounted unstained, or were stained with Mayer's haemalum; sometimes the sections were counterstained with eosin. Fresh eyes were examined after being teased out in 0·66 % saline.

Structure of the normal eye

The structure of an ommatidium of a normal (wild-type) imago of *Calliphora erythrocephala* is shown diagrammatically in Fig. 1. Distally there is a corneal lens (*c.l.*) and beneath that lies the pseudo-cone (*p.*), at the base of which there are four small cells with obvious nuclei (*c.c.*); these are the cone cells, and the nuclei correspond to those designated 'Semper's nuclei' by Eltringham (1919) although Lowne (1893–5) considers that 'Semper's nuclei' is a term used by different authors for different cell nuclei. Encircling the cone cells and the pseudo-cone there are two large flattened cells, each with a nucleus in the basal region. These are the *primary pigment cells* (*p.p.*). Lowne calls them the pigment cells of the cone and says that they are variable in number, two being the rule, but that he had occasionally seen three and that 'the whole structure of the ommatea indicates the probable existence of four'. Two is the number of these cells described for *Vanessa urticae* by Eltringham (1919) and for *Drosophila* by Johannsen (1924) and others, and that is the number always found in my preparations of *Calliphora erythrocephala*. Proximal to the base of the pseudo-cone, and extending inwards to the basement membrane (*b.m.*), is the rhabdom which is surrounded by the retinulae (*r.*). The number of retinulae is not easily determined. There are six retinulae with nuclei lying at the same level, in the enlarged distal ends of the cells (6 *r.n.*), but there is a seventh retinula of which the nucleus

lies a short distance proximal to those of the other six (7 *r.n.*). About one-fifth of the length from the proximal end of the ommatidium there is an eighth nucleus which appears to belong to an eighth retinula (8 *r.n.*), thus making eight retinulae in all. In *Drosophila* there is disagreement as to the number of retinulae and rhabdomeres. Johannsen (1924) says there are six normal and one displaced retinulae, or seven in all. Wolsky & Huxley

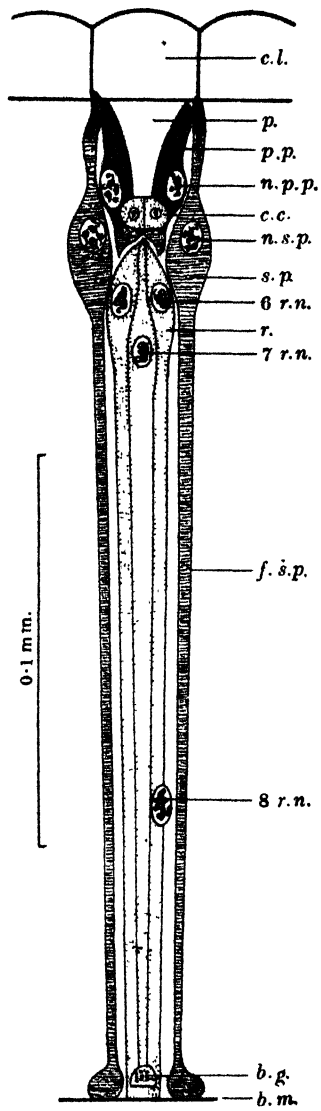

Fig. 1. Semi-diagrammatic representation of the structure of an ommatidium of a wild-type eye of *Calliphora erythrocephala*. *c.c.* cone cells; *c.l.* corneal lens; *b.g.* pigment granules at base of retinulae; *b.m.* basement membrane; *f.s.p.* fringe of secondary pigment cell; *n.p.p.* nucleus of primary pigment cell; *n.s.p.* nucleus of secondary pigment cell; *p.* pseudo-cone; *p.p.* primary pigment cell; *r.* retinula; 6 *r.n.* one of the six retinulae nuclei at this level; *s.p.* secondary pigment cell; 7 *r.n.* nucleus of seventh retinula; 8 *r.n.* nucleus of eighth retinula. The cross-hatching represents brown pigment granules. The horizontal shading represents red pigment granules.

(1936) say there are eight retinulae and eight rhabdomeres; Chevais (1937) gives the number of retinulae as six, but Hertweck (1931) and Pilkington (1942) state that there are six normal and two displaced retinulae and seven rhabdomeres, the eighth retinula being much reduced and functionless according to Hertweck. The arrangement of the retinulae described by Pilkington for *Drosophila* is very similar to that now shown to exist in *Calliphora erythrocephala*. Lowne (1893–5) depicts only the eighth retinular nuclei and calls them nuclei of the 'unpigmented cells of the sheath of the rhabdome', of which he

says there is a single nucleus in the imago, but in an earlier stage 'there are usually several nuclei at its extremities'. Dietrich (1908), however, states that eight retinular nuclei are always present, and that in *C. erythrocephala* there are six nuclei at one level and two displaced nuclei.

Surrounding the retinulae there are about 6–9 secondary pigment cells (*s.p.*). These cells are thick at the level of the tips of the retinulae, and in this thick part the nuclei are situated (*n.s.p.*). The secondary pigment cells send sheathing processes inwards as far as the basement membrane (*f.s.p.*) and outwards between and beyond the primary pigment cells so that their distal extremities separate the bases of adjacent corneal lenses. Where they meet the basement membrane the secondary pigment cells are enlarged, and probably it is these enlarged basal regions that Lowne described as 'inner pigment cells'. Dietrich (1908), in a figure of an ommatidium of *C. erythrocephala*, shows primary and secondary pigment cells but no 'inner' or 'basal' pigment cells.

The existence of a third series of pigment cells, 'basal pigment cells', is also a matter of dispute in *Drosophila*. Johannsen (1924) describes such cells as being present, but Cochrane (1936–7) considers that the thickened proximal ends of the secondary pigment cells may have been described as *basal* pigment cells, and agrees with Hertweck (1931) that basal pigment cells are not present. Pilkington (1942), however, states positively that they are present and 'are clearly seen in sections cut either longitudinally along the ommatidia, or transversely just above the basement membrane'. In imagines of *Calliphora* it is very difficult to determine if nucleated cells are present in this region owing to the dense masses of pigment granules present in the proximal parts of the secondary pigment cells; but study of sections of pupal eyes, before pigment is developed, shows conclusively that no cells are present in the place where 'basal' or 'inner' pigment cells have been described. This view is confirmed by the examination of sections of imaginal mutant eyes in which, owing to the absence of pigment, the structure and relationship of the various cells is much clearer. Owing to the fact that the secondary pigment cells are shared between neighbouring ommatidia, it is difficult to determine how many are related to an ommatidium, but as a rule nine of these cells impinge on each ommatidium.

The pigment of the eye is composed of granules of two colours, brown and red. The primary pigment cells of the imago contain dense brown pigment except in the basal regions surrounding the cone cells where it is admixed with red granules. The secondary pigment cells contain mostly red granules, except at the distal tips where there are dense accumulations of brown granules. The enlarged basal ends of the secondary pigment cells contain very dense masses of red granules. Within the rhabdom, at the base, there is a group of bright brown granules, the exact location of which is obscure but which appear to lie within a vacuole or cavity at the base of the retinulae.

In addition to the definite granular pigment, a yellow pigment is also present, but it is diffuse and is present in the retinulae and not in the pigment cells. This yellow colour becomes more intense with the age of the fly. According to Johannsen (1924) there is a similar yellow, non-granular pigment in the retinulae of *Drosophila*.

Development of eye pigment in pupae

To trace the time of the formation of pigment, pupae were incubated at 24° C. and examined at various ages.

The first appearance of pigment occurs at the fifth day of pupal life (126 hr.) when the

eyes are biscuit yellow, but microscopically the pigment appears to be diffuse in both primary and secondary pigment cells, and is not in granules. At 6 days of pupal life (150 hr.) the eyes are a rich yellow-brown, but as yet no granules can be detected microscopically. The eyes are brown in another 24 hr. (7 days), and the pigment cells now contain definite uniformly sized brown granules. The amount of brown pigment increases throughout the eighth and ninth days, but no red pigment appears until within a few hours of emergence which is at about 240 hr. (10 days) at 24° C. Immediately after emergence there is much red pigment in granules in the secondary pigment cells, but the pigment in the primary pigment cells remains brown except at the base, surrounding the cone cells, where red granules are also present. The change from brown to red pigment takes place very rapidly, as no red pigment is present 24 hr. before emergence but it is well developed in the newly emerged imago. Twenty-four hours after emergence the amount of red pigment in the secondary pigment cells has increased, and brown pigment granules are present in the tips of these cells which extend between the facets, and in the enlarged basal regions adjacent to the basement membrane. The primary pigment cells have dense masses of brown granules with red granules in their basal regions. A yellow, diffuse, non-granular pigment develops in the retinulae. The group of pigment granules at the base of the rhabdom retain their golden brown colour throughout.

In general sequence the development of eye pigmentation in *Calliphora* is similar to that in *Drosophila* as described by Schultz (1935) and by Cochrane (1936, 1936–7). Pigment formation is divisible into early and late phases. The early phase begins with the formation of yellow pigment, and the yellow is rapidly replaced by brown or tan pigment. The late phase occurs shortly before emergence, and consists of a great increase in the amount of pigment deposited and in the development of the definitive colour of the eye of the imago. In *Calliphora* the onset of the late phase is relatively later than in *Drosophila* and red pigment does not appear until just before emergence. In *Drosophila* the change from tan to red occurs at about 108–112 hr. after pupation, and the late phase of pigment deposition corresponds to an increase in both yellow and red pigments and sets in about 120 hr. after pupation, but emergence does not occur until 144 hr. (Cochrane, 1936).

THE STRUCTURE OF THE MUTANT EYE

Macroscopically the eyes of newly emerged mutant females are almost white or slightly yellowish, but as the flies age the eyes gradually darken to an apricot tinge. There is no further darkening beyond the apricot tinge, even in very old flies. The mutation is limited to the female sex and the eyes of males are always normal in appearance (Tate, 1947).

Microscopical examination of the eye of mutant imagines and of developing pupae of the mutant strains has shown that except for the absence of pigment granules the morphology of the eye does not differ from that of the normal eye. The pigment cells are fully formed and are present in the normal number, and have the same relation to the other elements of the ommatidia as in normal eyes. Although no pigment is formed in the pigment cells in the mutant eye, the diffuse non-granular yellow pigment that develops in the retinulae of normal eyes after emergence, develops also in the mutant eye, and it is this which gives the yellowish colour to such eyes and which is responsible for the gradual darkening of the eye with age to the apricot tinge. Cochrane (1938) has described a similar darkening of the eyes with age of 'buff' females of *Drosophila pseudo-obscura*. Thus the white-eyed mutant of *Calliphora* resembles the white-eyed mutants of *Drosophila*

(Cochrane, 1936) in that there is complete suppression of both early and late phase pigment deposition, although the eyes are fully developed and both primary and secondary pigment cells are present in the usual numbers.

Summary

1. The structure of the ommatidia of the eye of *Calliphora erythrocephala* is very similar to that of *Drosophila*.

2. Primary and secondary pigment cells are present, but the basal pigment cells described by Lowne could not be identified, and probably dense groups of granules in the basal expansions of the secondary pigment cells were mistaken for separate cells.

3. At 24° C. the onset of pigment formation in eyes during pupal life is at about 72 hr.; and the sequence is yellow, brown and red, but the late phase deposition of pigment, and the appearance of red granules, does not occur until a few hours before emergence, which at 24° C. is at 240 hr. after puparia formation.

4. The white-eyed mutant of *Calliphora erythrocephala*, which is sex-limited and appears only in the female, has eyes normal in structure, and with the full complement of both primary and secondary pigment cells, but there is complete suppression of the formation of all pigment granules.

REFERENCES

Chevais, S. (1937). Sur la structure des yeux implantés de *Drosophila melanogaster*. *Arch. Anat. micr.* **33**, 107–42.

Cochrane, F. (1936). Observations on eye-colour development in *Drosophila pseudo-obscura*. *J. Genet.* **32**, 183–7.

Cochrane, F. (1936–7). An histological analysis of eye pigment development in *Drosophila pseudo-obscura*. *Proc. Roy. Soc. Edinb.* **57**, 385–99.

Cochrane, F. (1938). Three new eye-colour mutations in *Drosophila pseudo-obscura*. *J. Genet.* **36**, 1–9.

Dietrich, W. (1908). Die Facettenaugen der Dipteren. *Z. wiss. Zool.* **92**, 465–559.

Eltringham, H. (1919). Butterfly vision. *Trans. R. Ent. Soc. Lond.* 1919–20, pp. 1–49.

Hertweck, H. (1931). Anatomie und variabilität des Nervensystems und der Sinnes-organe von *Drosophila melanogaster* (Meigen). *Z. wiss. Zool.* **139**, 559–663.

Johannsen, O. A. (1924). Eye structure in normal and mutant Drosophilas. *J. Morph.* **39**, 337–46.

Lowne, B. T. (1893–5). *The Anatomy, Physiology, Morphology and Development of the Blow-fly* (Calliphora erythrocephala). London: R. H. Porter.

Pilkington, R. W. (1942). Facet mutants of *Drosophila*. *Proc. Zool. Soc. Lond.* Ser. A, **111**, 199–222.

Schultz, J. (1935). Aspects of the relation between genes and development in *Drosophila*. *Amer. Nat.* **69**, 30–54.

Tate, P. (1947). A sex-linked and sex-limited white-eyed mutation of the blow-fly (*Calliphora erythrocephala*). *J. Genet.* **48**, 176–91.

Wolsky, A. & Huxley, J. S. (1936). The structure of the non-faceted region in the bar-eye mutants of *Drosophila*, and its bearing on the analysis of genic action upon arthropodan eyes. *Proc. Zool. Soc. Lond.* pp. 485–9.

AN IMPROVED GENETICAL MAP OF PUNNETT'S '**B**' CHROMOSOME IN THE SWEET PEA, *LATHYRUS ODORATUS* L.

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Introduction

It is common knowledge that statistics has been a close and helpful associate of genetics throughout its advancement. Bateson (1909, p. 318), attributing the success of Mendel primarily to the statistical nature of his investigations, alludes to Mendel's following remarks, 'Those who survey the work done in the department will arrive at the conviction that among all the numerous experiments made, not one has been carried out to such an extent and in such a way as to make it possible to determine the number of different forms under which the offspring of hybrids appear or to arrange these forms with certainty according to their separate generations or definitely to ascertain their statistical relations', and observes, 'It is to the clear conception of these three primary necessities that the whole success of Mendel's work is due....' The fairly recent statistical methods, e.g. the proper use of χ^2 and the analysis of variance, have revolutionized the planning of controlled experiments and the interpretation of their results. More particularly for the present paper, the methods of applying the theory of estimation based on the 'Method of Maximum Likelihood' are likely also to be valuable in the field of genetics.

A characteristic feature of such statistics is the ease with which they enable different bodies of data, either wholly or partially alike, to be combined to make improved estimates of parameters. The combination is made through a statistic known as 'Information', which also serves as a measure of the success of experiments in terms of the effort, time and expenses involved; and this enables improvements to be devised if necessary. This point is cogently elucidated by Mather (1938). Such statistics are, therefore, a valuable aid to the modern experimenter who often works under pressure and without adequate facilities.

Soon after the rediscovery of Mendel's laws, Bateson & Punnett undertook a comprehensive programme of work on the genetics of the sweet pea (*Lathyrus odoratus* L.). Among several other characters they studied (1) dark-light axil, (2) fertile-sterile (in Darwin's terms 'contabescent') anthers and (3) normal-cretin flower, which, on being studied in pairs, led to the discovery of the phenomenon of linkage.

Bateson & Punnett (1911) tried to explain linkage by postulating what they called 'Reduplication of gametes' in the germ tract. They and Punnett (1913) have described the hypothesis in detail. Punnett (1913) made earnest attempts to interpret the abnormal segregations observed in associated characters including those referred to above on this basis; but it is evident from the numerous doubts and difficulties he encountered that he was not satisfied with the results. Later, both because the reduplication hypothesis had no factual basis, and also since the 'Chromosome theory' of heredity propounded by Morgan and his associates gave overwhelming evidence of the linear arrangement of genes and their linkage in chromosomes, he seems to have acquiesced in the genetical map produced by Bridges (1914) in respect of the characters in question. He named the chromosome as the '**B**' chromosome (Punnett, 1923, 1932), and the genes responsible for the characters

as $\mathbf{B}_1$, $\mathbf{B}_2$ and $\mathbf{B}_3$ respectively. It must, however, be pointed out with regard to Bridges's map that it was, as he himself puts it, only 'illustrative', and this for two important reasons. First, the data, coming as they did from F_2 segregations; were not very adequate; and secondly, the method used for analysis, viz. Yule's coefficient of association, was 'the least unsatisfactory' available at the time. Thirdly, in the light of present-day knowledge the recombination percentages may not directly represent linear distances between genes as shown in his map. Fortunately, there are now opportunities to remedy all these defects. Punnett has in his records a large collection of unused data (compiled up to 1928) on the characters, which he has kindly made available for use. Fisher's new method of estimating linkage by 'scoring' (1946*b*), admirably suited to such type of work, together with Kosambi's formula for conversion of recombination percentages into linear distances, are also at hand. It is, therefore, thought fit to present in this paper an improved map of Punnett's '**B**' chromosome.

Review of methods of estimation

Ever since linkage was discovered by Bateson & Punnett attempts have been made by various workers, both statisticians and geneticists, to evolve formulae and methods for estimating the degree of association between characters. Amongst these may be mentioned methods developed by Yule, Collins, Emerson, Haldane, Takezaki, Kappert, Owen, Fisher & Balmukand, and Immer. All of these, however, fall under one or the other of the four categories discussed by Fisher (1946*a*), the only method efficient for all types of problems of estimation being the Method of Maximum Likelihood. However, it is considered essential to review briefly the methods employed by Bateson & Punnett on the one hand and by Bridges on the other, and compare them with Fisher's scoring system, first, to justify the choice of the scoring method in this paper, and, secondly, to show why revision of the map with this new device has been considered worth while.

The practice that Bateson & Punnett followed was more or less empirical. They assumed the following types of gametic series:

	Double dominant	Single dominant	Double recessive
Coupling	$(n-1)$*	1	$(n-1)$
Repulsion	1	$(n-1)$	1

generating zygotic series of the kind:

Coupling	$3n^2(2n-1)$	$(2n-1)$	$n^2-(2n-1)$
Repulsion	$(2n^2+1)$	(n^2-1)	1

Theoretical expectations were derived on this basis for certain selected gametic ratios, e.g. 1:3:3:1, 1:7:7:1, 1:15:15:1, 1:31:31:1, etc. Frequencies observed in progenies were compared by eye with the series of such theoretical expectations to see to which of the gametic series they conformed.

This method, while it very roughly indicated the intensity of linkage, could not have been anything more than one of approximation for obvious reasons. No wonder, therefore, that the inefficient estimates arrived at created difficulties in reconciling the behaviour of different progenies even in the same cross.

The method adopted by Bridges was, as previously stated, Yule's coefficient of asso-

* $(n-1):1$ or $n:1$ were supposed to be the gametic ratios brought about through alternating periclinal and anticlinal cell divisions in gametogenesis.

ciation,* which belongs to the category of the 'Coefficient of correlation' advocated by Takezaki (1925). Immer (1931) has discussed at length the efficiency of the 'Coefficient of correlation'. He concludes: 'The correlation method is fairly efficient in the coupling phase and for loose linkage in repulsion. For close linkage in repulsion it is not efficient.' He also draws attention to the inaccuracy of 'the error formula as used by Takezaki, which is based on the incorrect method of treating the fourfold table of phenotypic frequencies as a normal frequency surface'. The coefficient of association being even slightly inferior to the coefficient of correlation (Yule, 1912) is not therefore very efficient for estimation.

Fisher's (1946*b*) scoring method adopted in this paper is but the method of maximum likelihood in a form more convenient for use. Its efficiency is therefore assured. Its advantageous features, in addition to those of the Method of Maximum Likelihood, are:

(i) The scores are linear functions of the observed frequencies and additive; as such, 'the efficiently weighted combination of different lots of data is arrived at simply by adding their scores', thus making the combination an easy operation.

(ii) The aggregate score of a progeny is zero at the correct value of the estimate. As will further be seen, this property is very useful in detecting the type of mating.

(iii) The Information, which is the sampling variance of the score, while being essentially the same in nature as in the Maximum Likelihood method, serves, in this system, two useful objects, viz.:

(*a*) It indicates how far from or near to the trial value is the correct value of the parameter, the score at the trial value divided by its Information giving the correction factor leading towards the correct estimate.

(*b*) The square of the aggregate score of a progeny divided by its Information is its χ^2 for one degree of freedom. When several families or groups of families, say k in number, are pooled together, their homogeneity can be easily tested by adding their individual χ^2's derived in this way and subtracting therefrom the χ^2 for the totals, the remainder being χ^2 for $k-1$ degrees of freedom for heterogeneity as in any ordinary case. When different kinds of groups, each with many sub-groups, are combined, the χ^2 can be analysed into components as in the case of hierarchical classification.

(iv) The method includes a novel feature, based on Kosambi's formula, for improving the estimate of a smaller segment by taking account of information from bigger segments containing it. This should interest anyone who wishes to take maximum advantage of all data at his disposal. In view of the fact that geneticists have been so far estimating recombination percentages of any segment from data collected on that segment alone, this would definitely constitute *an advance in the estimation of linkage.*

There is no other method known at present commanding such unique efficiency and manifold advantages. This fact, together with the peculiar nature of the data, which include even families of unknown mating types in different filial generations, have favoured its choice for estimation.

MATERIAL AND METHODS

As stated above, the material used is Punnett's data of segregation for three characters in the sweet pea, viz. (i) dark-light axil, (ii) fertile-sterile anthers and (iii) normal-cretin flowers. The estimate of the recombination percentage of a fourth character (iv) purple-

* Yule's formulae (1900) for the coefficient of association and its error are

$$Q=\frac{(AB\times ab)-(Ab\times aB)}{(AB\times ab)+(Ab\times aB)} \quad \text{and} \quad E_Q=\frac{1-Q^2}{2}\sqrt{\frac{1}{AB}+\frac{1}{Ab}+\frac{1}{aB}+\frac{1}{ab}}.$$

maroon with no. (i) above from only one family of 155 F_2 plants (Punnett, 1923) has also been indicated at the end, though not made use of in locating the gene on the map. The first three characters have been repeatedly described by Bateson & Punnett. But, with a view to making this article self-contained and for facility in reading it, it has been thought proper briefly to reproduce their description below:

(i) *Dark-light axil.* 'Of the plants with white and coloured flowers in the sweet pea those with white (true whites have pale seed coats) have light (i.e. green) axils, whereas those with coloured flowers may have either dark or light axils.'

(ii) *Fertile-sterile (contabescent) anthers.* 'Contabescent anthers are sterile. Their chromosomes divide normally up to the reduction division when they form shapeless knots and fail to divide. Plants with sterile anthers are normal and fertile on the female side.'

(iii) *Normal-cretin flower.* 'The "Cretin" is a small deformed flower with varying structure of petals. In such a flower the standard fails to become elevated, the keel is cleft distally so that anthers are partially protruded, while the stigma sticks out far beyond the petals and is carried on in the line of the carpels instead of being abruptly bent at right angles to them as in the normal flower.'

(iv) *Purple-maroon flower colour.* The terms are self explanatory.

The data are presented in the Appendix, and are in all on 169 families consisting of 14,652 plants studied over a period of 25 years, from 1904 to 1928.* On considering families observed for three characters separately for every pair, their number is increased to 225 comprising 19,306 plants. They consist of 138 F_2 and 87 later (F_3 onwards) progenies. Classified according to character pairs they stand as given in Table 1.

Table 1. *Classification of plants according to character pairs, type of linkage, and families*

	Coupling		Repulsion	
Character pair	No. of families	No. of plants	No. of families	No. of plants
Dark-light axil	73	6635	35	4414
Fertile-sterile anthers	28	2239	21	1440
Normal-cretin flower	16	792	52	3786
Total	117	9666	108	9640

The number of plants observed (19,306) is nearly three times as large as that utilized by Bridges (6704), and should, on any consideration, be thought sufficient to give correct estimates of cross-over percentages.

It will, however, be seen that a large proportion of families belong to generations beyond F_2. Besides, even in the case of several F_2 families, the nature of the mating could not be made out from the records. Since it is not ordinarily possible to detect in such generations the type of mating, coupling or repulsion, of parents from which a progeny has been derived when segregation is on the border-line, one may wonder as to how they could all be rightly grouped under the two categories with confidence. In fact, this was one of the main difficulties in Punnett's (1913) classification of certain dubious families. For example, in the **dfN** × **DFn** mating (Table 11)† he put the four F_4 progenies, viz. 113/'13,114/'13, 117/'13 and 123/'13, in line with the two F_2 progenies, 52/'13 and 53/'13. As can be seen from their present classification (vide Appendix) the progenies 114/'13 and 117/'13 had been misclassified so far as character pairs **F-N** and **D-N** were concerned. Instead of being grouped under 'coupling' they were put with the rest of the F_2 and F_4

* These include all the families presented by Bateson, Saunders & Punnett (1905, 1908) and Punnett (1913). The later data were tabulated from Punnett's records kept at the Department of Genetics, Cambridge.

† In Punnett (1913, Table XI) this has been misprinted as **DfN** × **DFn**.

families under 'repulsion'. It is for this reason that his anticipation of repulsion between **N** and **F** and **N** and **D** was not realized for the whole group; indeed, the balancing effect of the mixture of 'coupling' and 'repulsion' families made him further suspect a normal segregation of 9 : 3 : 3 : 1. The fact that they were in coupling, and could not be otherwise, is proved by their very low χ^2's and homogeneity with the rest of the families in their present setting. Similarly, progenies 71/'12 and 91/'12 in the mating **Nf** × **nF** (Table 9), though classed separately, were suspected to indicate, both individually and together, a normal segregation. Difficulty was also experienced in identifying with confidence the families 47/'13 and 136/'13 (Table 10), though they were put under 'coupling'. All these families have fitted in the present groupings so well that there is little doubt left about the types of mating they came from.

The correct allocation of the mating type to every family has been rendered possible by the feature of the scoring method whereby the aggregate score of a family is zero at the correct value of the estimate. As a corollary of this characteristic it follows that a body of data scored at 50% for the appropriate mating gives a positive score, whereas when scored for the wrong one gives a negative one. This serves as a very useful means to detect the mating when one is in doubt about it. This method has been invariably used for the entire classification of families in the third and subsequent filial generations, and surprisingly enough all the eighty-seven and more families so classified have accommodated themselves very well in the respective groups in which they were put, as can be seen from their low (non-significant) χ^2's (see Appendix).

After tabulation, totals were struck of all progenies falling under each category, and the scoring method was further employed to work out recombination percentages. The *modus operandi* of estimation may be briefly described as follows:

If c represents the recombination percentage and N the number in the progeny, the expectations, in terms of c in a dihybrid intercross, of the three classes, viz. (1) double dominants, (2) single dominants, and (3) double recessives are, respectively,

$$\frac{N}{4}(3-2c+c^2), \quad \frac{N}{4}(2c-c^2), \quad \frac{N}{4}(1-c)^2;$$

and the differentials of the expected frequencies are

$$-\frac{N}{2}(1-c), \quad +\frac{N}{2}(1-c), \quad -\frac{N}{2}(1-c).$$

The scores are obtained by dividing the differentials by expectations and changing sign, as below:

$$\frac{2(1-c)}{2+(1-c)^2}, \quad -\frac{2(1-c)}{2c-c^2}, \quad \frac{2}{(1-c)} \quad \text{for coupling;}$$

$$-\frac{2c}{2+c^2}, \quad \frac{2c}{1-c^2}, \quad -\frac{2}{c} \quad \text{for repulsion.}$$

The amount of Information per individual, which is the summation of squares of scores multiplied by expectations over the three classes is, in this case,

$$\frac{2+4(1-c)^2}{(2c-c^2)(3-2c+c^2)} \quad \text{for coupling,} \qquad \text{and} \qquad \frac{2+4c^2}{(1-c^2)(2+c^2)} \quad \text{for repulsion.}$$

To facilitate scoring Fisher has prepared tables of scores for various recombination percentages from 1 to 50 for intercross progenies. The scores are given at unit intervals and can be conveniently interpolated by the usual method (Fisher & Yates, 1938

'Introduction—Table XXXIII—Random Numbers—Interpolation'). Except in cases where extraordinary accuracy is aimed at a four-point interpolation is deemed satisfactory. For such an interpolated value the information is given by the formula

$$100D+\{1-\tfrac{1}{6}(1-3\theta^2)\,\delta^2 u_{x+1}\}-\{1-\tfrac{1}{6}(1-3\phi^2)\,\delta^2 u_x\},$$

where D is the positive difference between scores at x and $x+1$, θ = fraction beyond x, $\phi=1-\theta$, δ^2 = central second difference.

The estimation is done through a series of trials beginning with 50%. The total score of a family at any trial value divided by its Information gives the correction factor to arrive at a more correct value. This procedure is repeated until a satisfactory estimate giving a score almost zero* is reached. With experience and proper judgement the number of such trials can be reduced to only about two or three. Primary estimates made in this way for the three segments $\mathbf{B}_1$–$\mathbf{B}_2$, $\mathbf{B}_1$–$\mathbf{B}_3$ and $\mathbf{B}_2$–$\mathbf{B}_3$ and the scores and Information thereon are given below:

Segment	Recombination percentages	Score	Information
$\mathbf{B}_1$–$\mathbf{B}_2$	3·03	−85·5042894	
$\mathbf{B}_1$–$\mathbf{B}_3$	28·00	−113·5092077	
$\mathbf{B}_2$–$\mathbf{B}_3$	28·42	−0·0601295	7216·16941

At this stage, Kosambi's formula is employed to improve the estimates of the two smaller segments, $\mathbf{B}_1$–$\mathbf{B}_2$ and $\mathbf{B}_1$–$\mathbf{B}_3$, with information obtained from the larger segment, $\mathbf{B}_2$–$\mathbf{B}_3$, which contains them both. Kosambi's formula enunciating the relationship between two smaller segments (say y_1 and y_2) with a bigger one containing them (y_{12}) is

$$y_{12}=\frac{y_1+y_2}{1+4y_1y_2}.$$

This relationship also provides factors with which to multiply the score of the larger segment to obtain its contribution of scores to the smaller ones. The factors are obtained from the following corollaries of the above formula:

$$\frac{\partial y_{12}}{\partial y_1}=\frac{1-4y^2{}_2}{(1+4y_1y_2)^2},\quad \frac{\partial y_{12}}{\partial y_2}=\frac{1-4y_1{}^2}{(1+4y_1y_2)^2}.$$

The following are the actual calculations in this case:

Assuming $\mathbf{B}_1$–$\mathbf{B}_2$ to be 3·03 and $\mathbf{B}_1$–$\mathbf{B}_3$ to be 28·000, $\mathbf{B}_2$–$\mathbf{B}_3$ by Kosambi's formula would be 30·0115288. The approximate score at this value of the estimate is +114·7872849, i.e. (−0·0601295 + 7216·16941 × 0·015915288),†

$$\frac{\partial y_{12}}{\partial y_1}=0{\cdot}6420812,\quad \frac{\partial y_{12}}{\partial y_2}=0{\cdot}9319977.$$

The scores and Information for the two segments are:

	Score	Information
	$\mathbf{B}_1\mathbf{B}_2$	
From $\mathbf{B}_1$–$\mathbf{B}_2$	−85·5042894	222256·4513561
From $\mathbf{B}_2$–$\mathbf{B}_3$	+73·7027575	2954·8783446
Total	−11·8015318	225211·3297007
	$\mathbf{B}_1\mathbf{B}_3$	
From $\mathbf{B}_1$–$\mathbf{B}_3$	−113·5092077	9259·3783681
From $\mathbf{B}_2$–$\mathbf{B}_3$	+106·9814855	6225·7170433
Total	−6·5277222	15485·0954114

* When improvement of the estimate of a smaller segment with the help of information from a larger segment is aimed at, as is done in this paper, it may be preferable to bring the score approximately to such a figure as would serve to nullify the score that would be derived for the segment in question from the larger segment.

† This is the difference between the provisional (0·2842) and the calculated (0·300115288) estimate of y_{12}.

The product term of Information for the two segments is obtained by multiplying the information for $\mathbf{B}_2$–$\mathbf{B}_3$ at 30·0115288 by the product of the two factors derived above:

$$= 7690{\cdot}047928 \times 0{\cdot}642812 \times 0{\cdot}9319977$$
$$= 4289{\cdot}0834065.$$

The adjustments of estimates for the two segments p for $\mathbf{B}_1$–$\mathbf{B}_2$ and q for $\mathbf{B}_1$–$\mathbf{B}_3$ are now calculated by solving the following simultaneous equations:

$$225211{\cdot}3297007p + 4289{\cdot}0834065q = +11{\cdot}8015318,^*$$
$$4289{\cdot}0834065p + 15485{\cdot}0954114q = + 6{\cdot}5277222,^*$$

whereby $p = 0{\cdot}00004$ and $q = 0{\cdot}0041$.

The new values obtained by applying the adjustments are

$$\mathbf{B}_1\mathbf{B}_2 = 3{\cdot}034;\ \mathbf{B}_1\mathbf{B}_3 = 28{\cdot}041;\ \mathbf{B}_2\mathbf{B}_3 = 30{\cdot}052 \text{ (newly calculated).}$$

On repeating the procedure twice over the following scores and Information were obtained for the two smaller segments:

	Score	Information
	$\mathbf{B}_1\mathbf{B}_2$	
From $\mathbf{B}_1\mathbf{B}_3$	−75·5293950	221830·969681
From $\mathbf{B}_2\mathbf{B}_3$	+75·5292207	2945·253238
Total	−0·0001743	224776·222919
	$\mathbf{B}_1\mathbf{B}_3$	
From $\mathbf{B}_1\mathbf{B}_2$	−109·7725205	9248·513214
From $\mathbf{B}_2\mathbf{B}_3$	+109·7780603	6221·910222
Total	+0·0055398	15470·423436

Here the scores are very nearly reduced to zero. It is, therefore, not worth while to work out further adjustments. Standard errors of estimates are calculated by the following formulae:

$$V(y_1) = \frac{I_{22}}{\Delta}, \quad V(y_2) = \frac{I_{11}}{\Delta}.$$

$$V(y_{12}) = \left(\frac{\partial y_{12}}{\partial y_1}\right)^2 \frac{I_{22}}{\Delta} - 2\frac{\partial y_{12}}{\partial y_1}\frac{\partial y_{12}}{\partial y_2}\frac{P}{\Delta} + \left(\frac{\partial y_{12}}{\partial y_2}\right)^2 \frac{I_{11}}{\Delta}.$$

I_{11} = total Information for y_1.
I_{22} = total Information for y_2.
P = product term of Information to be calculated as above.
$\Delta = I_{11}I_{22} - p^2$.

On arriving at final estimates, map lengths in 'centimorgans' were found by referring to Fisher & Yates (1938, Table No. XII, Transformation of 'r' to 'z' Interclass).

THE RESULTS

The entire results obtained are set out in Table 2.

Table 2. *Recombination percentages, scores, Information and map lengths for the three segments in Punnett's* '**B**' *chromosome*

Segment	Recombination percentage	Map length (cm.)	Score	Information
$\mathbf{B}_1\mathbf{B}_2$	3·0359 ± 0·21	3·04 ± 0·21	−0·0001743	224776·222919
$\mathbf{B}_1\mathbf{B}_3$	28·0410 ± 0·81	31·70 ± 1·18	−0·0055398	15470·423436
$\mathbf{B}_2\mathbf{B}_3$	30·0535 ± 0·75	34·74 ± 1·17	+117·8159978	7166·402000

The standard error (S.E.) of map length (x) corresponding to the R.P. (y) is calculated by using the formula

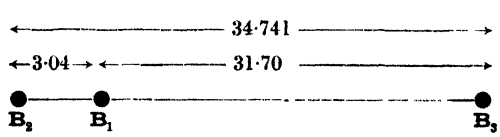
$$\text{S.E. } (x) = \text{S.E.}(y)/(1 - 4y^2).$$

* Written after reversing the signs.

Table 3. *Estimates of recombination percentages arrived at by Punnett, Bridges and the author*

Segment		Punnett	Bridges	Author
B_1–B_2	C	6·25	4·4	3·04
	R	Not ascertained	4·9	
B_1–B_3	C, R	25·00	28·0, 27·0	28·04
B_2–B_3	C, R	25·00	33·0, 28·0	30·05

It will be seen from the table that even Bridges's values are somewhat different from those estimated by the writer, though the differences are not very striking to a casual observer. The value of such differences can be appreciated only when one considers the effect they would have in genetical experiments. For example, in a repulsion intercross the expectation of a double recessive with 4·4 % R.P. would be 1 in 2056, while that with 3·04 % R.P. would be 1 in 4328, not an inconsiderable difference when one is confronted with the question of obtaining such a plant. Apart from its utility or consequences a genuine difference, however small, should be welcome as a scientific fact, especially when it is brought out with the support of enormous convincing evidence. Looking at the figures from this point of view the new estimations appear justified. The map drawn on the basis of the new estimates is given below:



After obtaining the estimates, χ^2 tests were made, first, to judge whether it was appropriate to use Kosambi's formula in this case, and secondly, to test the homogeneity of the data used to derive the estimates.

The first test is essential since Kosambi's formula may not be applicable to all types of cases, and unless it is proved that the estimates are in agreement with the formula they would not be reliable. The χ^2's for this test were calculated by squaring the score of each segment and dividing by the amount of Information at the final estimated value. Their total is the χ^2 to judge the conformity of the estimates to the formula. The figures are produced in Table 4. The value of χ^2, though high for one degree of freedom, is not sufficiently high (being less than 3·84, the five per cent value) to contradict the formula.

Table 4. *Values of χ^2 to test agreement with Kosambi's formula*

Segment	Score	Information	χ^2
B_1B_2	−75·529395	221830·969681	0·026
B_1B_3	−109·7725205	9248·513214	1·303
B_2B_3	117·8159978	7166·402000	1·937
Total			3·266

The test of homogeneity was done in the usual manner, χ^2 for each family at the final estimated value having been calculated exactly as in the above case. The figures of χ^2 for individual families and probabilities are given in the Appendix. The χ^2 of heterogeneity between mating types, and between families in each type are set out in Table 5.

Table 5. *χ^2's and probabilities for heterogeneity between mating types, and between families within mating types*

Factor pair	Between mating types χ^2	Between mating types P	Mating type	Between families within mating types D.F.	χ^2	P
B_1–B_2	0·00	>0·99	Coupling	72	82·55	0·19*
			Repulsion	34	27·09	0·80*
B_1–B_3	0·37	0·5~0·7	Coupling	27	27·77	0·3~0·5
			Repulsion	20	15·59	0·7~0·8
B_2–B_3	0·09	0·7~0·8	Coupling	15	8·18	0·9~0·95
			Repulsion	51	48·13	0·60*

* Probability in terms of the normal deviate obtained by the formula $\sqrt{2\chi^2} - \sqrt{(2n-1)}$ for one degree of freedom.

The test of homogeneity is very important in this case, as the number of progenies handled is very large, and they come from different filial generations and mating types. It is much more so because, as previously stated, the mating types in a majority of cases have been fixed on the basis of their total scores at 50% level. Since the method is totally unconventional it is possible that its efficacy may be doubted, particularly with the data involving families which Punnett (1913) had found very heterogeneous and irregular, unless proved by this test.

It is very striking, however, that every mating type, even with its large number of families, is clearly homogeneous. The same is also the case with the two mating types for each pair of characters. Further, a glance at the χ^2's of individual families would show that they are almost unanimous in indicating the cross-over values estimated in the respective cases. Only one family, viz. 22/'15 (B_1B_2 repulsion) shows a χ^2 beyond the 1% level of probability. The reasons for including it are:

Family no. 22/'15. The high χ^2 in this case is the result of one plant having appeared in the double recessive class instead of the expected (0·006) fraction of a plant. There are in all 4414 plants in the B_1B_2 repulsion group and, with estimated R.P. of 3·03%, just one double recessive plant is expected to occur. Since the group consists of thirty-four families in all it has to appear in some one of these and, when it does, it is *but to be expected* that it will upset the balance of that particular family. When it is borne in mind that the double recessive plant holds the balance between an accurate and inaccurate estimate in such a case of close linkage in repulsion it would be seen that it would have been inadmissible to leave out the family.

Out of all the progenies found in the records five B_1–B_2 progenies appeared to be irregular. All these belong to the coupling group as can be easily made out from the distribution of their frequencies, and as confirmed by their scores at 50%. Yet they were far too different from other sister progenies entered in the Appendix, as is indicated by their very high χ^2's at the recombination percentage estimated from the rest of the families, i.e. 3·10%. Their particulars are set out in Table 6.

Table 6. *Particulars of five B_1–B_2 (coupling) families which were originally found to be irregular*

Serial no.	Family ref. no.	Frequencies B_1B_2	B_1b_2	b_1B_2	b_1b_2	χ^2 at 3·10%
1	351/'6G	14	—	4	7	13·29
2	96/'22	50	1	11	10	46·35
3	57/'27	185	3	49	66	199·31
4	58/'27	314	7	100	152	449·54
5	75/'27	69	1	18	40	54·91

None of these five came from the lot that Punnett found difficult to classify. On the other hand, every one of those that were not amenable to grouping and interpretation is, with grouping altered where necessary, in perfect harmony with the rest in the particular class wherein it is placed, in indicating the estimated recombination value.

In the case of the last four progenies the segregation for the 'fertile-sterile' character was more or less in conformity with the expected 3 : 1 ratio, but that for 'dark-light' was unsatisfactory. Closer examination of the classification showed that an additional factor 'self-marbled', located in the '*F*' chromosome, was also involved. It was further noticed that all 'marbled' plants had been classified only as 'light' and that the segregation between 'self-dark', 'self-light' and 'marbled-light' bore a relation of 9 : 3 : 4. It was clear, therefore, that some of the 'marbled' plants contained the gene for 'Dark axil', and that it could not manifest itself due to the absence of the gene for 'self' colour. On such a hypothesis, after excluding the 'marbled' plants, the remaining ones should indicate a recombination of about 3%, which they did very well. These families were, therefore, included in the $\mathbf{B}_1$–$\mathbf{B}_2$ coupling group, and now show very low χ^2's (vide Appendix) at the joint estimate of 3·04%.

In the case of the first family, viz. 351/'6G, the difficulty has remained unsolved. The segregation for the two factors under study is as follows:

dark-fertile	dark-sterile	light-fertile	light-sterile
14	0	4	7

The ratio for dark-light is 9 : 7, while that for fertile-sterile is 3 : 1. Clearly there is an additional gene involved in the segregation of the dark and light characters. The estimate made on the basis of these individual segregations is 16·74%, which is very different from the estimate of 3·0% arrived at from other progenies. The actual classification by Punnett is as below:

		fertile	sterile
Full purple:	dark	7	—
	light	1	4
Light purple:	dark	—	—
	light	1	1
Full red:	dark	4	—
	light	2	2
Pale red:	dark	3	—
	light	—	—

It can be seen from the distribution that there is no consistent absence of either 'dark' or 'light' plants in either 'full' or 'pale (or light)' colours—purple or red, which might serve to eliminate the effect of the second gene on the dark-light segregation. The progeny also being very small, its exclusion will not involve appreciable loss of Information; so it has been omitted from consideration.

In conclusion it may be stated that every one of the dubious families is satisfactorily classified in the present analysis. The striking homogeneity found in the large amount of data studied speaks highly both for the remarkable accuracy of the data and for the efficiency of the scoring system of estimation.

The purple-maroon ($\mathbf{B}_4$) *character*

It may be stated in passing that the family 50/'22, which Punnett studied (1923) and found difficult to interpret, indicates a recombination value of 19·3% between the characters, dark-light axil and purple-maroon flower colour. For want of more data it is not possible to give a definite estimate of it. Neither can the position of the gene be located, as the character has not been studied with others in the chromosome.

SUMMARY

An improved genetical map of Punnett's 'B' chromosome in the sweet pea is presented on the basis of Punnett's data recorded from 1904 to 1928 on the characters (1) dark-light axil, (2) fertile-sterile anthers and (3) normal-cretin flowers. The data have been classified and analysed by Fisher's scoring method estimating linkage. The procedure of estimation has been briefly illustrated.

Prof. R. C. Punnett, F.R.S., has very generously left his original notebooks at the Department of Genetics, Cambridge, for the use of students. I am very thankful to him for the data drawn from his books and also for his encouragement in this work. It is difficult to express my deep gratitude to Prof. R. A. Fisher, Sc.D., F.R.S., at whose instance the study was undertaken and under whose guidance it was accomplished. I am also thankful to Dr D. S. Falconer and Mr T. C. Carter, M.A., for kindly going through the paper and making useful suggestions.

REFERENCES

BATESON, W. (1909). *Mendel's Principles of Heredity.* Camb. Univ. Press.

BATESON, W. & PUNNETT, R. C. (1911). On the gametic series involving reduplication of certain terms. *J. Genet.* **1**, 293–302.

BATESON, W., SAUNDERS, E. R. & PUNNETT, R. C. (1905). Experimental studies in the physiology of heredity. *2nd Rep. Evol. Comm. Roy. Soc.* pp. 80–99.

BATESON, W., SAUNDERS, E. R. & PUNNETT, R. C. (1908). Experimental studies in the physiology of heredity. *4th Rep. Evol. Comm. Roy. Soc.* pp. 2–16.

BRIDGES, C. B. (1914). The chromosome hypothesis of linkage applied to cases in sweet peas and *Primula*. *Amer. Nat.* **48**, 524–34.

IMMER, F. R. (1931). The efficiency of the correlation coefficient for estimating linkage intensities. *Amer. Nat.* **65**, 567–72.

FISHER, R. A. (1946*a*). *Statistical Methods for Research Workers.* 10th ed. Edinburgh: Oliver and Boyd.

FISHER, R. A. (1946*b*). A system of scoring linkage data with special reference to pied factors in mice. *Amer. Nat.* **80**, 568–78.

FISHER, R. A. & YATES, F. (1938). *Statistical Tables.* Edinburgh: Oliver and Boyd.

MATHER, K. (1938). *The Measurement of Linkage in Heredity.* Methuen.

PUNNETT, R. C. (1913). Reduplication series in sweet peas. *J. Genet.* **3**, 77–103.

PUNNETT, R. C. (1923). Linkage in the sweet pea. *J. Genet.* **13**, 101–23.

PUNNETT, R. C. (1925). *Lathyrus odoratus. Bibliogr. genet.* **1**, 69–82.

PUNNETT, R. C. (1927). Linkage groups and chromosome number in *Lathyrus*. *Proc. Roy. Soc.* B, **102**, 236–8.

PUNNETT, R. C. (1932). Further studies of linkage in the sweet pea. *J. Genet.* **26**, 97–112.

TAKEZAKI, Y. (1925). On a new method of computation of the cross-over value from F_2 zygotic series. *J. Genet.* **3**, 105–13.

YULE, G. U. (1900). On the association of attributes in statistics. *Philos. Trans.* A, **194**, 257–319.

YULE, G. U. (1912). On the methods of measuring association between two attributes. *J. Roy. Statist. Soc.* **75**, 579–652.

APPENDIX

Table showing the segregation of the characters, dark-light axil (B_1) *and fertile-sterile anthers* (B_2)

Coupling

Family no. From the *Rep. Evol. Comm. Roy. Soc.* 1905, p. 91	B_1B_2	B_1b_2	b_1B_2	b_1b_2	Total	χ^2 (at 3·0359)
141/'4F	121	5	2	39	167	0·82
142/'4F	25	2	—	9	36	0·75
143/'4F	29	—	—	11	40	1·33
144/'4F	43	—	2	17	62	0·00
181/'4G	23	—	2	12	37	0·51
120/'5F	40	1	3	9	53	4·14
316/'5G	41	1	2	20	64	0·40
317/'5G	53	1	1	14	69	0·00
342/'6G	50	1	—	11	62	0·27
348/'6G	20	1	1	5	27	1·93
349/'6G	22	1	2	6	31	4·88
350/'6G	57	3	1	21	82	0·89
352/'6G	28	2	1	6	37	3·64
353/'6G	7	1	0	6	14	0·52
354/'6G	22	1	2	5	30	5·34
358/'6G	17	—	—	8	25	0·94
1/'9	15	—	1	8	24	0·05
2/'9	49	2	2	11	64	2·66
62/'10	62	1	1	18	82	0·06
67/'11	114	2	3	30	149	0·13
102/'12	69	3	3	24	99	3·06
104/'12	24	—	—	5	29	0·72
108/'12	32	—	—	11	43	1·36
109/'12	7	—	—	1	8	0·17
52/'13	14	—	—	7	21	0·81
53/'13	19	1	1	7	28	1·57
113/'13*	23	3	—	15	41	1·95
114/'13*	40	—	2	10	52	0·19
117/'13*	38	1	1	10	50	0·22
123/'13*	25	—	—	7	32	0·91
71/'21	27	1	—	9	37	0·01
72/'21	318	5	2	110	435	3·08
73/'21	100	2	1	28	131	0·16
96/'21	71	—	1	22	94	1·16
99/'21	38	—	—	14	52	1·71
100/'21	46	—	2	5	53	0·34
73/'22	15	—	1	6	22	0·17
75/'22	4	1	—	9	14	0·27
76/'22	25	1	—	9	35	0·01
77/'22	34	3	—	15	52	1·13
92/'22*	41	1	4	17	63	4·81
93/'22*	58	2	1	21	82	0·09
96/'22*	19	1	—	7	27	0·03
97/'22*	50	1	—	9	60	0·19
99/'22*	52	2	—	10	64	0·05
60/'24*	24	—	—	9	33	1·09
64/'24*	22	—	—	8	30	0·98
65/'24	31	1	3	10	45	5·33
43/'25	27	3	—	6	36	3·82
44/'25	110	—	2	18	130	0·46
45/'25	53	2	—	16	71	0·00
46/'25	101	1	2	26	130	0·12
47/'25	106	1	4	39	150	0·03
48/'25	86	1	1	32	120	0·85
49/'25	127	2	2	40	171	0·24
50/'25	147	—	2	44	193	2·39
96/'25	131	2	5	32	170	1·00
	70	—	—	20	90	2·59

* Families in generations later than F_2.

Family no.	B_1B_2	B_1b_2	b_1B_2	b_1b_2	Total	χ^2 (at 3·0359)
97/'25	103	1	6	61	171	0·20
98/'25	67	—	—	17	84	2·28
51/'27	148	2	7	45	202	1·55
52/'27	136	5	1	53	195	0·00
53/'27	108	3	1	35	147	0·04
54/'27	114	2	3	39	158	0·00
57/'27	185	3	3	46	237	0·06
58/'27	314	7	7	106	434	0·06
63/'27	113	—	2	33	148	1·27
64/'27	103	1	—	33	137	2·39
65/'27	157	1	3	50	211	0·86
66/'27	39	1	—	11	51	0·15
71/'27	87	2	1	31	121	0·15
73/'27	60	—	4	23	87	0·64
75/'27	69	1	2	32	104	0·59
73	4865	95	106	1569	6635	0·025
				Deviation χ^2 (1)		0·025
				Heterogeneity χ^2 (72)		82·545
				Total		82·570

Repulsion

Family no.	B_1B_2	B_1b_2	b_1B_2	b_1b_2	Total	χ^2 (at 3·0359)
84/'8	95	45	44	—	184	0·03
95/'8	31	12	21	—	64	0·02
97/'8	37	29	31	—	97	0·07
106/'8	53	17	22	—	92	0·01
107/'8	214	95	102	—	411	0·07
108/'8	43	22	29	—	94	0·03
109/'8	81	50	58	—	189	0·09
110/'8	72	27	39	—	138	0·02
111/'8	110	55	49	—	214	0·04
112/'8	177	73	99	—	349	0·07
56/'10	27	17	18	—	62	0·03
60/'10	126	59	69	—	254	0·06
48/'12	49	28	27	—	104	0·03
48/'13	129	55	55	—†	239	0·03
49/'13	24	17	15	—	56	0·03
50/'13	17	14	11	—	42	0·02
51/'13	50	28	25	—	103	0·03
30/'14	27	13	19	—	59	0·02
31/'14	62	24	35	—	121	0·02
32/'14	58	27	18	—	103	0·01
33/'14	40	17	20	—	77	0·01
22/'15*	85	41	30	1	157	26·14
79/'15	104	50	46	—	200	0·04
80/'15	121	50	60	—	231	0·04
54/'16	15	12	6	—	33	0·01
55/'16	59	20	19	—	98	0·03
59/'16	31	7	11	—	49	0·00
127/'19	19	8	13	—	40	0·01
57/'20*	55	20	20	—	95	0·06
58/'20*	63	19	34	—	116	0·01
75/'20*	6	7	3	—	16	0·01
79/'20*	17	5	6	—	28	0·00
87/'20*	71	26	50	—	147	0·04
92/'21*	42	31	27	—	100	0·05
93/'21*	31	10	11	—	52	0·03
35	2241	1030	1142	1	4414	0·00
				Deviation χ^2 (1)		0·00
				Heterogeneity χ^2 (34)		27·09
				Total		27·09

* Families in generations later than F_3.

† Two plants in this class have been eliminated on discovering a remark by Punnett in the record book that they were 'not good plants', as without them the family conforms to the degree of linkage indicated by the rest of the families.

Table showing the segregation of the characters, dark-light axil (B_1) *and normal-cretin flower* (B_3)

Coupling

Family no.	B_1B_3	B_1b_3	b_1B_3	b_1b_3	Total	χ^2 (at 28·041)
48/'13	151	33	31	26	241	1·30
49/'13	37	4	5	10	46	2·60
50/'13	27	4	5	6	42	0·20
51/'13	67	11	8	17	103	2·41
114/'13*	32	7	7	4	50	0·91
117/'13*	22	3	4	3	32	0·00
30/'14	32	8	12	7	59	2·43
31/'14	76	10	21	14	121	0·25
32/'14	71	14	8	10	103	0·02
33/'14	52	5	9	11	77	1·33
22/'15*	104	22	14	17	157	0·00
36/'15*	44	7	5	6	62	0·17
39/'15*	15	4	1	1	21	0·18
55/'15*	20	3	8	2	33	2·27
57/'15*	14	1	9	3	27	2·01
59/'15*	30	—	4	3	37	1·48
60/'15*	14	3	4	1	22	1·39
79/'15	127	27	23	23	200	0·24
80/'15	140	31	30	30	231	0·45
54/'16	26	1	5	1	33	0·00
55/'16	69	10	14	5	98	1·02
59/'16	32	6	6	5	49	0·08
127/'19	24	3	7	6	40	0·00
57/'20*	61	14	7	13	95	0·21
79/'20*	21	1	3	3	28	0·75
87/'20*	82	15	23	27	147	0·15
60/'24*	19	3	2	6	30	1·58
64/'24*	22	10	8	5	45	4·92
28	1431	260	283	265	2239	0·58
				Deviation χ^2 (1)		0·58
				Heterogeneity χ^2 (27)		27·77
				Total		28·35

* Families in generations later than F_2.

Repulsion

Family no.	B_1B_3	B_1b_3	b_1B_3	b_1b_3	Total	χ^2 (at 28·041)
52/'13	13	7	7	1	28	0·13
53/'13	19	7	12	3	41	4·52
113/'13*	25	15	11	1	52	0·06
119/'13*	48	27	22	3	100	0·17
123/'13*	20	8	8	1	37	0·17
34/'14	54	21	14	3	92	1·93
35/'14	22	7	9	1	39	0·24
36/'14	20	5	3	—	28	0·01
37/'14	52	24	21	1	98	0·33
38/'14	199	63	79	6	347	0·24
25/'15*	31	16	20	1	68	0·50
29/'15*	31	11	19	3	64	1·71
30/'15*	22	9	10	—	41	0·64
38/'15*	15	6	6	—	27	0·33
40/'15*	21	7	3	1	32	1·16
41/'15*	41	12	8	1	62	0·48
56/'15*	39	24	19	1	83	0·72
58/'15*	20	7	16	—	43	1·42
61/'15*	11	4	10	1	26	0·08
58/'20*	71	11	33	1	116	0·00
75/'20*	9	4	2	1	16	1·81
21	783	295	332	30	1440	1·09
				Deviation χ^2 (1)		1·09
				Heterogeneity χ^2 (20)		15·59
				Total		16·68

* Families in generations later than F_2.

Table showing the segregation of the characters fertile-sterile anthers (B_2) *and normal-cretin flower* (B_3)

Coupling

Family no.	B_2B_3	B_2b_3	b_2B_3	b_2b_3	Total	χ^2 (at 30·0535)
76/'12*	90	30	20	34	174	0·22
91/'12*	20	5	4	3	32	0·23
38/'13*	16	9	8	12	45	0·08
39/'13*	27	9	17	14	67	1·23
40/'13*	20	4	7	9	40	0·38
41/'13*	12	6	6	9	33	0·00
47/'13*	23	5	4	4	36	0·00
114/'13*	32	7	7	4	50	0·51
117/'13*	22	3	4	3	32	0·03
136/'13*	6	1	1	1	9	0·02
16/'15*	18	2	3	2	25	0·06
58/'20*	91	6	13	6	116	0·73
75/'20*	7	2	4	3	16	0·33
60/'24*	19	3	2	6	30	2·14
64/'24*	25	9	5	6	45	0·36
17/'28*	28	1	6	7	42	2·10
16	456	102	111	123	792	0·24
				Deviation χ^2 (1)		0·24
				Heterogeneity χ^2 (15)		8·18
				Total		8·42

* Families in generations later than F_3.

Repulsion

Family no.	B_2B_3	B_2b_3	b_2B_3	b_2b_3	Total	χ^2 (at 30·0535)
5/'9	51	33	30	1	115	2·89
72/'11	26	10	14	1	51	0·05
73/'11	21	12	12	1	46	0·18
74/'11	24	8	9	—	41	0·37
75/'11	22	4	4	2	32	5·15
76/'11	30	5	12	1	48	0·27
77/'11	78	32	43	3	156	0·26
78/'11	59	24	15	—	98	0·64
79/'11	25	15	12	2	54	0·11
70/'12*	37	34	22	1	94	3·33
71/'12*	22	9	9	2	42	1·15
80/'12*	8	1	6	—	15	0·29
82/'12*	12	8	9	—	29	1·68
86/'12*	17	6	10	—	33	0·79
87/'12*	49	16	11	1	77	0·09
88/'12*	11	6	7	—	24	0·99
90/'12*	24	13	11	—	48	1·35
92/'12*	11	5	7	—	23	0·80
99/'12*	12	7	3	—	22	0·36
43/'13*	20	4	7	1	32	0·66
45/'13*	37	10	9	2	58	1·80
46/'13*	18	7	7	2	34	2·02
48/'13	133	49	51	8	241	2·16
49/'13	25	17	14	—	56	2·54
50/'13	19	13	9	1	42	0·11
51/'13	48	27	27	1	103	1·81
52/'13	13	7	7	1	28	0·04
53/'13	17	6	14	4	41	6·57
113/'13*	27	15	9	1	52	0·03
123/'13*	19	8	9	1	37	0·02
30/'14	32	12	14	1	59	0·02
31/'14	74	23	23	1	121	0·04
32/'14	52	24	27	—	103	2·75
33/'14	44	16	17	—	77	0·88
22/'15*	81	34	37	5	157	0·48
79/'15	105	45	45	5	200	0·06
80/'15	123	58	47	3	231	0·69
54/'16	20	1	11	1	33	0·49
55/'16	65	13	18	2	98	1·09

Repulsion (cont.)

Family no.	B_2B_3	B_2b_3	b_2B_3	b_2b_3	Total	χ^2 (at 30·0535)
57/'16	13	4	5	—	22	0·18
58/'16	20	7	7	—	34	0·29
59/'16	31	11	7	—	49	0·15
127/'19	24	8	7	1	40	0·27
128/'19	49	22	23	2	96	0·05
57/'20	51	24	17	3	95	0·48
79/'20	19	4	5	—	28	0·00
80/'20	37	14	17	1	69	0·12
81/'20*	43	24	24	1	92	1·42
82/'20*	26	15	13	1	55	0·28
83/'20*	58	25	31	3	117	0·00
87/'20*	80	41	25	1	147	1·07
121/'21*	47	17	26	1	91	0·60
52	2009	853	855	69	3786	1·79
				Deviation χ^2 (1)		1·79
				Heterogeneity χ^2 (51)		48·13
				Total		49·92

* Families in generations later than F_2.

THE GENETICS OF BLACKARM RESISTANCE

VI. TRANSFERENCE OF RESISTANCE FROM *GOSSYPIUM ARBOREUM* TO *G. BARBADENSE*

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Introduction

Despite a fairly comprehensive survey of the various cotton species of the world, complete immunity to blackarm disease (*Bact. malvacearum*, Sm.) has been found only in the two species of 'Old World' ($n = 13$) cottons, *Gossypium arboreum* and *G. herbaceum*. Of these, *G. arboreum* includes many immune types, and, since the advantages of immunity over resistance are obvious, an investigation of the genetic nature of this immunity was undertaken, (1) to identify the gene or genes responsible, (2) to transfer any such genes to Sakel (*G. barbadense*), and (3) to determine the relationship between the *arboreum* gene or genes and the 'New World' resistance genes $\mathbf{B}_1$, $\mathbf{B}_2$ and $\mathbf{B}_3$.

This paper deals with the transference of the Asiatic resistance gene $\mathbf{B}_4$ to Sakel. The genetics of resistance within *G. arboreum* will be published in due course.

Previous work

Knight & Clouston (1939, 1941) and Knight (1944*a*) have shown that, in the New World allotetraploid cottons, resistance to blackarm, where present, is due, in *G. hirsutum*, to the gene $\mathbf{B}_2$ very occasionally accompanied by a weak gene $\mathbf{B}_1$, and in *G. hirsutum* var. *punctatum* (= *G. punctatum*, Sch. & Thon.) either to $\mathbf{B}_2$ or to $\mathbf{B}_3$, or to both genes acting additively. It was further shown that $\mathbf{B}_2$ and $\mathbf{B}_3$ are linked and have a 32% crossover value. Finally, $\mathbf{B}_1$ is closely linked with (or possibly identical with) the allele of one of a pair of duplicate genes which together control normality of growth as opposed to a dwarfing described under the name 'dwarf-bunched' (Knight, 1947*a*). On a system of grading in which '0' = immunity and '12' full (Sakel) susceptibility, but from which grade '11' is missing, $\mathbf{B}_1\mathbf{b}_1$ and $\mathbf{B}_1\mathbf{B}_1$ confer '10·1' resistance when transferred to Sakel,

$\mathbf{B_2b_2}$ and $\mathbf{B_2B_2}$ confer grade '7', $\mathbf{B_3b_3}$ gives '7·1'–'8·1' and $\mathbf{B_3B_3}$ '4·1'–'7·1' resistance. This system of grading resistance is fully defined in Part I of this series and is redefined and illustrated in Part IV.

In Part I of this series, over 160 varieties of cotton are classified on their blackarm resistance and this number is further extended by Knight (1943, 1944*a*, *b*, 1946*a*, 1947*b*). These results and further data hitherto unpublished are summarized in tabular form below:

Species*	Reaction to blackarm disease
(*a*) Diploid ($n=13$) types:†	
Gossypium thurberi	Susceptible
G. harknessii	Resistant to susceptible
G. klotzschianum	Resistant
G. klotzschianum var. *davidsonii*	Resistant
G. armourianum	Resistant
G. raimondii	Resistant
G. aridum	Resistant
G. stocksii	Susceptible
G. somalense	Resistant
G. herbaceum	Immune to susceptible
G. arboreum	Immune to susceptible
G. anomalum	Highly resistant
G. sturtii	Susceptible
(*b*) Allotetraploid ($n=26$) types:	
G. hirsutum	Resistant to susceptible
G. hirsutum var. *punctatum*	Highly resistant to susceptible
G. hirsutum var. *marie-galante*	Susceptible
G. barbadense	Mainly susceptible
G. barbadense var. *darwinii*	Susceptible
G. tomentosum	Susceptible

* The nomenclature adopted throughout this paper is that used by J. B. Hutchinson in Part I of *The Evolution of 'Gossypium'*, Oxford University Press; by Hutchinson, J. B., Silow, R. A. and Stephens, S. G.

† It should be borne in mind that many of the samples of the wild species of cotton have originated at some stage from single plants, consequently these results do not necessarily apply to the species as a whole since this has not in all cases been adequately sampled.

G. barbadense var. *darwinii* has previously been reported as resistant (Knight, 1944*b*, 1946*b*) but this record was made on an accession received from abroad under the name *G. darwinii* and subsequently identified as *G. hirsutum* var. *punctatum* (Knight, 1947*b*).

Preparation of inoculum and technique of infecting the plants

Knight & Clouston (1939) described the method used for preparing inoculum and for infecting plants evenly with blackarm. Knight (1946*b*) has since simplified the original method and the following account is quoted from this source. 'Experience has shown, however, that perfectly even infection can be obtained by using an inoculum made by soaking 5 lb. of air-dried diseased leaves in 40 gal. of water, instead of the 10 lb. originally advocated. The infected leaves are soaked in water for 1 hr. and then continuously crushed and stirred for a further hour, after which the suspension is strained through sacking and used immediately. "Solo-sprayers" are used for spraying and the jet is directed at the plant from ground-level upwards. Spraying is done twice a day for two successive days so that, on the present technique, each plant receives four sprayings with suspension compared with the six previously advocated.'

TRANSFERENCE OF RESISTANCE FROM *G. ARBOREUM* TO *G. BARBADENSE*

Description of strains

Multani (Sanguineum), strain NT12/30, belongs to *G. arboreum* race *bengalense*. It is a red-leaved, sympodial type which, even after repeated spraying with inoculum, is usually immune to blackarm disease. In certain seasons a small proportion of plants show grade '1', '2' and even '3' symptoms but, owing to the way in which anthocyanin pigment increases the apparent size both of small lesions and insect punctures, it has never been certain that these plants were actually attacked by blackarm.

Domains Sakel (*G. barbadense*) is an established Egyptian commercial type which needs no further description here. Like all 'Egyptian' cottons it shows full (*barbadense*) susceptibility to blackarm, i.e. grade '12'.

F_1 of Multani × Sakel

In 1940–1 winter a number of Multani ($n=13$) plants were treated with colchicine in the cotyledon stage and later planted out in an isolated place. Although typical colchicine 'symptoms' were produced on the early leaves of some of these plants these abnormalities disappeared in the later leaves and the plants all appeared to be typical $n=13$ Multani.

During the coolest part of the season these plants were regularly pollinated, before sunrise, with Sakel ($n=26$) pollen. The Multani flowers were not emasculated, since setting of a reasonable quantity of seed per boll ensures against undue boll shedding. Seed from these 'hybrid' bolls was delinted with concentrated sulphuric acid. The largest seeds were then picked out, it being assumed that these would contain triploid ($2n=39$) embryos. These seeds proved, with one exception, to be of F_1 type and the exception gave rise to a tetraploid ($2n=52$) Multani plant.

This type of crossing has several times been repeated using various arboreum cottons, not previously treated with colchicine, as female parents. In no case were F_1 embryos found amongst the large seeds.* Furthermore, crosses between autotetraploid Multani female and Sakel male, yielded F_1 plants indistinguishable from those obtained in the original hybridization. It thus seems probable that some of the Multani plants used as female parents in 1940–1 winter contained mixoploid tissue of diploid/tetraploid type and that the resulting Multani × Sakel F_1 plants were autoallotetraploids of $2n=52$ composition carrying two Multani genoms and one Sakel genom, i.e. $2A_2+(AD)_2$. The actual composition of these F_1 plants must remain conjectural since the writer had, at the time, no facilities for making chromosome counts.

Twenty-five of these Multani × Sakel plants were raised. Only fifteen of them were graded for blackarm resistance since the remainder had been grown out-of-season when grading is difficult and often inaccurate. In the following season, a second F_1 family, consisting of four plants, was raised and examined for blackarm. This second F_1 was obtained by crossing tetraploid Multani female with Sakel male. Adding these two F_1's together gives the following distribution:

Blackarm grade							
'1'	'2'	'3'	'4'	'5'	'6'	'7'	'8'
5	5	2	3	3	.	.	1

* The writer has, however, twice seen F_1 plants from the reciprocal cross resulting from open pollination of 'Egyptian' types grown in isolated areas and belted by Multani.

First Sakel backcross

Using the F_1 of Multani × Sakel as male parent, 500 pollinations of emasculated Sakel flowers were made. No bolls set.

Using the F_1 plants as female parent, 15,000 pollinations with Sakel pollen were made. The F_1 proved self-sterile, hence its flowers were not emasculated, but pollination was carried out early in the morning during the coolest part of year. These pollinations yielded eighty-nine seeds, and these were sown in 1942–3 summer. Thirty-one plants were raised, two of which died as a result of ant attack. The remainder were graded for blackarm resistance with the following result:

Table 1. *Blackarm grading of first Sakel backcross*

Family no.	Parent grade	Blackarm grade								
		'3'	'4'	'5'	'6'	'7'	'8'	'9'	'10'	'12'
BA391/42	'2'	.	.	.	1	.	.	1	.	1
BA392/42	'4'	.	.	.	.	2	2	.	.	1
BA393/42	'5'	.	1	.	.	2	1	2	1	3
BA394/42	'1'	.	.	.	.	.	1	1	.	.
BA396/42	?	.	.	.	.	.	1	.	.	.
BA397/42	'2'	.	.	1	.	1	.	.	.	.
BA399/42	'5'	1	.	.	1	.	2	1	1	.
Totals		1	1	1	2	5	7	5	2	5

Second Sakel backcross

Using the first backcross plants as female parents, a large number of pollinations with Sakel pollen were made. Most of the plants in the first backcross progenies showed a high degree of sterility, but a small amount of second backcross seed was obtained and sown. Much of this seed failed to germinate, including, unfortunately, the seed from the only highly resistant (grade '3') plant which proved at all fertile.

The blackarm grading of these second backcross progenies is shown below (Table 2):

Table 2. *Blackarm grading of second Sakel backcross*

Family no.	Parent grade	Blackarm grade					
		'6'	'7'	'8'	'9'	'10'	'12'
BA61/43	'9'	.	.	1	.	.	.
BA289/43	'7'	.	1	.	.	.	1
BA290/43	'7'	1	.	1	.	.	4
Totals		1	1	2	.	.	5

BA61/43 is from BA391/42; BA289 and 290/43 are from BA393/42 (Table 1).

Third Sakel backcross

Of the four resistant plants in the second Sakel backcross, two showed moderate fertility, viz. those in families BA61/43 and BA289/43. All four were pollinated with Sakel pollen, but seed was obtained only from these two plants. The two resulting backcross progenies were examined for resistance with the following result (Table 3):

Table 3. *Blackarm grading of third Sakel backcross*

Family no.	Parent grade	Blackarm grade			
		'8'	'9'	'10'	'12'
BA61/44	'8'	5	.	.	5
BA416/44	'7'	1	.	.	1
Totals		6	.	.	6

BA61/44 is from BA61/43; BA416/44 is from BA289/43 (Table 2).

Fourth Sakel backcross

The third backcross plants showed reasonably good fertility and they were crossed in both directions with Sakel. The resistant plant in BA416/44 was equally effective in transmitting resistance whether it was the male or the female parent (in BA90/45, below, it was the male parent and in BA91/45, the female). One plant in BA61/44 was used as female parent and this gave rise to family BA485/45, below. Three other plants were selected in BA61/44 and these were used as male parents; they gave rise to families BA486/45, BA487/45 and BA414/44 (Table 4):

Table 4. *Blackarm grading of fourth Sakel backcross*

Family no.	Parent grade	Blackarm grade '6'	'7'	'8'	'9'	'10'	'12'
BA90/45	'8'	.	.	6	.	.	1
BA91/45	'8'	.	.	4	.	.	1
BA485/45	'8'	.	8	4	.	.	20
BA486/45	'8'	.	2	.	.	.	58
BA487/45	'8'	2	5	1	.	.	114
BA414/44	'8'	.	.	.	2	.	11

BA90 and 91/45 are from BA416/44; BA485–487/45 and BA414/44 are from BA61/44 (Table 3).

Since the reciprocal hybrids, BA90/45 and BA91/45, show a preponderance of resistant plants, it is evident that resistance did not, in this case, have any deleterious effect on the pollen carrying it. In the remaining four families, however, BA485/45 shows a distribution of twelve resistant plants to twenty susceptibles—a fair approximation to a 1 : 1 ratio in view of the small size of the family. BA486/45 and BA487/45 both show very distorted ratios, two resistant and fifty-eight susceptible in the one and eight resistant to 114 susceptible in the other. The female parent of BA485/45 was resistant whereas the resistant plants were the male parents of BA486/45 and BA487/45. The ratio, in the last family (BA414/44), of two resistant to eleven susceptible plants possibly represents a similar distortion, but the numbers are small and chance might equally well be the cause.

Fifth Sakel backcross

Five resistant plants in BA90/45 and three in BA91/45 (Table 4) were backcrossed to Sakel, using the latter as female parent. BA90 and 91/45 were sown in 1944–5 winter and the fifth backcross progenies from them were sown in 1945 summer. The distributions obtained in these backcross progenies are shown below (Table 5):

Table 5. *Blackarm grading of fifth Sakel backcross*

Family no.	Parent grade	Blackarm grade '6'	'7'	'8'	'9'	'10'	'12'	Totals Res.	Sus.
BA490/45	'8'	.	6	3	.	.	14	9	14
BA491/45	'8'	1	5	6	.	.	10	12	10
BA492/45	'8'	4	27	6	.	.	31	37	31
BA493/45	'8'	1	13	4	.	.	19	18	19
BA494/45	'8'	5	12	2	.	.	14	19	14
BA495/45	'8'	.	8	2	.	.	6	10	6
BA496/45	'8'	3	18	4	.	.	20	25	20
BA497/45	'8'	.	1	1	.	.	3	2	3
Totals		14	90	28	.	.	117	132	117
					Expected		(1 : 1)	124·5	124·5

BA490–495/45 are from BA90/45, and BA495–497/45 are from BA91/45 (Table 4).

From these figures it is evident that a single factor for blackarm resistance has been transferred from Multani to Sakel.

F_2 of fourth Sakel backcross

In the two winter-sown families, BA 90/45 and BA 91/45 (Table 4), all the plants were selfed after they had been used for backcrossing to Sakel, and these self-bred fourth backcross F_2 progenies were grown in 1945 summer. As in the late backcross progenies, clear segregation into 'resistant' and 'susceptible' (grade '12') was obtained (Table 6):

Table 6. *Blackarm classification of F_2 of fourth Sakel backcross*

Family no.	Observed		Expected (3 : 1)	
	Res.	Sus.	Res.	Sus.
BA716/45	37	16	39¾	13¼
BA717/45	3	2	3¾	1¼
BA718/45	8	1	6¾	2¼
BA719/45	27	8	26¼	8¾
BA721/45	57	18	56¼	18¾
BA723/45	14	9	17¼	5¾
Totals	146	54	150	50

BA716–721/45 are from BA90/45; BA723/45 is from BA91/45 (Table 4).

In conjunction with the distributions found in the second, third, fourth and fifth Sakel backcrosses, the above figures clearly prove that a single factor for resistance has been transferred from Multani to Sakel.

F_3 of fourth and fifth Sakel backcrosses

Among the resistant plants in the F_2 progenies of the fourth and fifth Sakel backcrosses, selections were made from the extremes of the range. Grade '6'–'7' represented the maximum resistance obtained and '9' the minimum. Progenies of these plants were classified for resistance with the results shown in Tables 7 and 8.

It will be seen from the progenies shown in these two tables that out of a total of forty-three grade '6'–'7' parent plants, twenty-four were homozygous and nineteen heterozygous for resistance against an expectation on a 1 : 2 basis of 14⅓ and 28⅔ respectively. For these totals $\sigma = 3{\cdot}091$ and P is approximately 0·003. Thus there is a significant tendency for the more resistant plants in F_2 to be homozygous. Similarly, an examination of the progenies from grade '9' parents shows that nine of these were homozygotes whilst twenty-four of them were heterozygotes. These figures do not differ greatly from expectation on a 1 : 2 basis of eleven and twenty-two respectively.

Treating these figures as a contingency table gives the following result:

	Homozygotes	Heterozygotes	Totals
Grade '6'–'7'	24	19	43
Grade '9'	9	24	33
Totals	33	43	76

$\chi^2 = 6{\cdot}191$. P lies between 0·01 and 0·02.

Clearly dominance is incomplete.

Table 7. *Blackarm classification of F_3 of fourth Sakel backcross*

(*a*) Progenies from heterozygotes

Family no.	Parent grade	No. of plants Res.	Sus.
BA71/46	'7'	20	8
BA76/46	'7'	27	9
BA78/46	'7'	25	10
BA82/46	'7'	23	5
BA85/46	'7'	32	4
BA86/46	'7'	23	5
BA88/46	'7'	21	10
BA90/46	'7'	22	7
BA504/46	'6'	24	6
BA658/46	'7'	22	5
BA660/46	'7'	30	5
BA72/46	'9'	22	8
BA73/46	'9'	25	11
BA74/46	'9'	17	5
BA75/46	'9'	27	11
BA499/46	'9'	31	9
BA500/46	'9'	33	6
BA502/46	'9'	31	11
BA527/46	'9'	32	8
BA528/46	'9'	33	9
BA530/46	'9'	31	10
BA661/46	'9'	16	6
BA662/46	'9'	14	4
BA663/46	'9'	22	4
Totals		603	176
Expected	(3 : 1)	584¼	194¾

(*b*) Progenies from homozygotes

Seven progenies from grade '6' parents, eleven from grade '7' parents and six from grade '9' parents proved to be homozygous for resistance. In size these homozygous families ranged from seventeen to forty-three plants.

Table 8. *Blackarm classification of F_3 of fifth Sakel backcross*

(*a*) Progenies from heterozygotes

Family no.	Parent grade	No. of plants Res.	Sus.
BA495/46	'7'	30	10
BA496/46	'7'	25	5
BA497/46	'7'	28	5
BA512/46	'7'	32	6
BA518/46	'7'	29	10
BA524/46	'7'	37	6
BA525/46	'7'	30	10
BA526/46	'7'	30	5
BA491/46	'9'	28	11
BA493/46	'9'	19	8
BA494/46	'9'	12	6
BA507/46	'9'	14	8
BA508/46	'9'	15	7
BA509/46	'9'	29	6
BA510/46	'9'	30	10
BA515/46	'9'	27	8
BA516/46	'9'	26	7
BA517/46	'9'	32	10
BA522/46	'9'	15	3
Totals		488	141
Expected	(3 : 1)	471¾	157¼

(*b*) Progenies from homozygotes

Six progenies from grade '7' parents and three progenies from grade '9' parents proved to be homozygous for resistance. In size these homozygous families ranged from twenty-eight to forty-two plants.

Check crosses with $\mathbf{B}_1$, $\mathbf{B}_2$ *and* $\mathbf{B}_3$

Heterozygous resistant plants from the fifth Sakel backcross progenies were crossed with a Sakel strain homozygous for $\mathbf{B}_2$, transferred from American Upland (*Gossypium hirsutum*). In F_2 the following distributions were obtained:

Table 9. *Blackarm grading of* F_2 *of check cross with* $\mathbf{B}_2\mathbf{B}_2$ *Sakel*

Family no.	Blackarm grade '3'	'4'	'5'	'6'	'7'	'8'	'9'	'10'	'12'	Totals Res.	Totals Sus.	Expected (15 : 1) Res.	Expected (15 : 1) Sus.
BA474/46	13	65	71	20	.	.	.	.	11	169	11	168·75	11·25
BA475/46	.	.	54	49	1	.	.	.	37	104	37	($\mathbf{B}_2$ only)	
BA476/46	7	49	60	26	1	.	.	.	15	143	15	148·12	9·88
BA477/46	.	.	106		.	.	.	.	27	106	27	($\mathbf{B}_2$ only)	
BA480/46	.	.	117		.	.	.	.	44	117	44	($\mathbf{B}_2$ only)	
Control 1	.	.	28	15	.	.	.	.	.	.	.	.	.
Control 2	.	7	61	13	1	.	.	.	.	.	.	.	.
Totals (BA474 and 476)	20	114	131	46	1	.	.	.	26	312	26	316·87	21·13

Note. Control 1 = $\mathbf{B}_2\mathbf{B}_2$ Sakel; Control 2 = Sakel of fifth backcross F_3 type homozygous for the Multani resistance gene.

Clearly $\mathbf{B}_2$ and the transferred *arboreum* gene occupy different loci.

In these families blackarm development was poor for climatic reasons, and this explains the unduly high resistance of the control types. Nevertheless, neither control contained grade '3', and grade '4' only represented a low proportion of control no. 2. It is evident, therefore, from the distributions obtained in BA474 and 476/46, that the *arboreum* gene and $\mathbf{B}_2$ show additive effect on a *barbadense* background.

Crosses between heterozygous resistant fifth backcross plants and a Sakel strain homozygous for $\mathbf{B}_3$ were grown out-of-season. The F_1 plants ranged in resistance from grade '4' to '7'. Five plants showing maximal resistance were selfed and their progenies yielded the following distributions:

Table 10. *Blackarm grading of* F_2 *of check cross with* $\mathbf{B}_3\mathbf{B}_3$ *Sakel*

Family no.	Blackarm grade '3'	'4'	'5'	'6'	'7'	'8'	'9'	'10'	'12'	Totals Res.	Totals Sus.	Expected (15 : 1) Res.	Expected (15 : 1) Sus.
BA481/46	.	56	74	29	14	7	.	.	8	180	8	176·25	11·75
BA482/46	.	33	91	33	22	14	.	.	16	193	16	195·90	13·06
BA483/46	1	43	94	28	11	4	.	.	14	181	14	182·85	12·19
BA484/46			88				.	.	6	88	6	88·13	5·88
BA485/46			66				.	.	3	66	3	64·69	4·31
Control 3	.	.	18	22	3	.	.	.	.	.	.	.	.
Control 2	.	7	61	13	1	.	.	.	.	.	.	.	.
Totals										708	47	707·82	47·19

Note. Control 3 = $\mathbf{B}_3\mathbf{B}_3$ Sakel, Control 2 = Sakel of fifth backcross F_3 type homozygous for the Multani resistance gene. Families BA484 and 485/46 were not graded in detail.

Clearly, from the above figures, the transferred Multani resistance factor and $\mathbf{B}_3$ occupy separate loci and show no linkage. Furthermore, these distributions indicate that the Multani gene has some additive value in conjunction with $\mathbf{B}_2$.

In the F_1 progenies of crosses between the heterozygous Multani factor and $\mathbf{B}_1\mathbf{B}_1$ Sakel, approximately 50% of the plants showed '10·1' ($\mathbf{B}_1$) resistance and the remainder showed

resistance similar to that conferred by the homozygous Multani gene. In F_2 the following distributions were obtained, in progenies derived from the more resistant plants:

Table 11. *Blackarm grading of F_2 of check cross with $\mathbf{B}_1\mathbf{B}_1$ Sakel*

Family no.	Blackarm grade								Totals		Expected (15 : 1)	
	'4'	'5'	'6'	'7'	'8'	'9'	'10·1'	'12'	Res.	Sus.	Res.	Sus.
BA486/46	2	66	43	7	3	.	24	9	145	9	144·38	9·63
BA487/46	4	76	56	10	7	.	18	11	171	11	170·63	11·38
BA488/46				195				15	195	15	196·88	13·13
BA489/46				197				15	197	15	198·75	13·25
BA490/46				195				17	195	17	198·75	13·25
Control 2	7	61	13	1	.	.	.	.	.	.	.	.
Totals									903	67	909·39	60·64

Note. Families BA488–490/46 were not graded in detail.

Thus the transferred Multani gene and $\mathbf{B}_1$ are independent. Moreover, $\mathbf{B}_1$ adds little if anything to the resistance conferred by the homozygous *arboreum* gene.

DISCUSSION

Two main points of interest arise out of this *arboreum-barbadense* transference of a blackarm resistance gene, viz. (*a*) the possible economic value of the gene itself, and (*b*) the economic possibilities of the method of transference used.

It has been shown that the new gene, $\mathbf{B}_4$, has additive properties in conjunction with either of the New World genes, $\mathbf{B}_2$ and $\mathbf{B}_3$. This is likely to be important since *immunity* (as distinct from *resistance*) has not as yet been either found or synthesized in the allotetraploid cottons. It is of considerable interest that this new gene, when transferred to the *barbadense* genom, segregates independently of the other three known genes. This fact cannot be regarded as evidence concerning the number of loci affecting blackarm resistance unless it is known that a high degree of gene homology exists between the Asiatic genom and the corresponding half of the New World genom. That gene homology exists in at least some cases is shown by Harland's (1935) successful transference of the *arboreum* gene $\mathbf{R}_2^{RS}$ to *Gossypium hirsutum* from a type similar to Multani. Harland showed the transferred gene to be homologous with the New World anthocyanin gene series.

There are three possible methods by which a gene (or genes) can be transferred from *G. arboreum* to *G. barbadense*: (*a*) the allotetraploid method, (*b*) the autotetraploid, and (*c*) straight transference via the triploid. In using the allotetraploid technique for transferring resistance to blackarm it would be necessary to start by crossing Multani with a fully susceptible diploid New World *Gossypium* such as *G. thurberi*. The F_1 of this cross would then have to be doubled with colchicine or another polyploiding agent.

The autotetraploid technique is illustrated by the transference of $\mathbf{B}_4$ recorded in this paper and the 'straight transference' method was used successfully by Harland (1935) when he transferred the *arboreum* gene $\mathbf{R}_2^{RS}$ to *G. hirsutum*.

Using Beasley's (1942) symbols for the various genoms involved, the initial F_1's in these three techniques can be represented as follows:

$$(a)\ A_2D_1(AD)_2,\quad (b)\ A_2A_2(AD)_2,\quad (c)\ A_2(AD)_2.$$

Of these three F_1's only the first is approximately 'balanced', but it is known that gene transference to cultivated allotetraploid cottons is possible from the A_2 genom to the (AD) genom in all three.

A full discussion of the merits of method (*a*) must await a critical examination of this method of transference as compared with method (*b*). The outstanding point (to the pure geneticist) in favour of method (*a*) is that it is known that accurate studies of gene homology can be made by this means. The disadvantages are (1) that it is not always easy to synthesize the required *arboreum*-New World tetraploid, and (2) that the complication of a second foreign genom is introduced.

The disadvantage of method (*c*) is the extreme difficulty that is experienced in making the triploid F_1 of $A_2(AD)_2$ composition. The writer has many times unsuccessfully tried to make this F_1.

Method (*b*) has certain advantages of its own: (1) It is simple to make the original autotetraploid by soaking *arboreum* seeds in 0·05 % colchicine for 48 hr. (2) This tetraploid shows good fertility if used as a female parent in crosses with *barbadense*. (3) Although the F_1 with *barbadense* lacks fertility this is not a difficult obstacle to overcome since a large F_1 can readily be made.

The best system is to grow a large F_1 in an isolated place (to avoid contamination with foreign pollen) and to pollinate open F_1 flowers with *barbadense* pollen without emasculation. Thus, for example, an F_1 of tetraploid *arboreum* × Sakel, consisting of 89 plants, was grown at Shambat this season. A single boy, working 1½ hr. a day throughout the cool season (4 months), was responsible for all pollination. From some 23,000 pollinations 209 seeds of first backcross type were obtained, but it should be noted that Pink and Egyptian bollworms had probably destroyed at least an equal number of seeds.

The use of a large F_1 population is essential in order to obtain a first backcross progeny of adequate size. In the present case, a backcross progeny of twenty-nine plants contained three plants showing a certain degree of fertility. There can be little doubt that by increasing the size of the backcross progeny one is automatically increasing the chance of finding fertile plants. Where a single known dominant (or partially dominant) gene is being transferred, the method is to grow a large first backcross progeny, eliminate all plants not carrying the required gene, and, in the remainder, to select the plants showing maximum fertility for further backcrossing.

It is of interest that hybridization between Asiatic and New World cottons seems to be less successful when American Upland is used than when *barbadense* is the New World parent. Thus the writer has crossed tetraploid Multani with two varieties of American Upland—XA 129 and BAR.SP 84. In both cases the F_1's died gradually, before the first flowers opened. Similarly, J. B. Hutchinson informs me that crosses between an *arboreum* × *thurberi* tetraploid and the American Upland type, U 4, do not grow more than a few inches high.

Summary

A strong, partially dominant gene governing resistance to blackarm disease (*Bact. malvacearum*) has been transferred from *Gossypium arboreum* to *G. barbadense* (Domains Sakel). The new gene, $\mathbf{B_4}$, segregates independently of $\mathbf{B_1}$, $\mathbf{B_2}$ and $\mathbf{B_3}$ and it shows additive effect in conjunction with $\mathbf{B_2}$ and with $\mathbf{B_3}$.

Three cytogenetically distinct techniques for transferring genes from Old World diploid to New World allopolyploid cottons are described and their relative merits discussed.

REFERENCES

BEASLEY, J. O. (1942). *Genetics*, **27**, 25–54.

HARLAND, S. C. (1935). The genetics of cotton. XII. *J. Genet.* **30**, 465–76.

KNIGHT, R. L. (1943). *Progr. Rep. Exp. Sta. Emp. Cott. Gr. Corp.* 1941–2.

KNIGHT, R. L. (1944 *a*). The genetics of blackarm resistance. IV. *J. Genet.* **46**, 1–27.

KNIGHT, R. L. (1944 *b*). *Progr. Rep. Exp. Sta. Emp. Cott. Gr. Corp.* 1942–3.

KNIGHT, R. L. (1946 *a*). *Progr. Rep. Exp. Sta. Emp. Cott. Gr. Corp.* 1944–5.

KNIGHT, R. L. (1946 *b*). Breeding cotton resistant to blackarm disease (*Bact. malvacearum*). *Emp. J. Exp. Agric.* **14**, 153–74.

KNIGHT, R. L. (1947 *a*). The genetics of blackarm resistance. V. *J. Genet.* **48**, 43–50.

KNIGHT, R. L. (1947 *b*). *Progr. Rep. Exp. Sta. Emp. Cott. Gr. Corp.* 1945–6.

KNIGHT, R. L. & CLOUSTON, T. W. (1939). The genetics of blackarm resistance. I. *J. Genet.* **38**, 133–59.

KNIGHT, R. L. & CLOUSTON, T. W. (1941). The genetics of blackarm resistance. II and III. *J. Genet.* **41**, 391–409.

THE ROLE OF MAJOR GENES IN THE EVOLUTION OF ECONOMIC CHARACTERS

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Introduction

The early Mendelians devoted much attention to the more obvious plant and animal characters, such as pigmentation, dwarfing and the like, and a wealth of information confirming Mendel's work was soon built up. The next stage—the application of genetics to economic ends—disclosed difficulties, and experience has shown that many economic characters do not give simple Mendelian ratios in hybridization. As a result of this, modern thought tends more and more to stress the importance of minor genes. This school of thought has been developed by Fisher (1930) and 'Student' (1933, 1934), and extended by Mather (1943*a*, *b*) with his polygene concept. The polygene school holds that quantitative characters are normally built up by the combined effect of a number of minor genes brought together in response to selection pressure. A second school, led by the Russian academician Lysenko, maintains that the whole Mendelian outlook is fallacious. Lysenko's (1946) theories, however, take so little account of established fact and scientific proof that for the purposes of this paper they may safely be disregarded. Studies of the response of commercial cottons to blackarm* attack (Knight & Clouston, 1939; Knight, 1944, 1946) have indicated that major genes are more important in the synthesis of resistant types than might have been supposed. From this a theory has been developed, and is set forth in this paper, of the significance of major genes in adaptive response.

Definitions

The terms 'major' and 'minor' genes are used throughout this paper as a convenient shorthand—clearly each must merge into the other so that no hard and fast line can be drawn between them. Major gene is used in the sense of a gene which, as compared with its allele (or weakest allele), produces a large effect. A minor gene, on the other hand, when compared with its allele produces an insignificant effect. Obviously there must be all degrees of gene 'size' from these major genes to the minor genes—a normal curve of gene 'size'—but it is the genes which produce large differences that are of outstanding

* Blackarm is a rain-borne disease caused by *Bacterium* (*Xanthomonas*) *malvacearum*.

interest to the applied geneticist. It is the aim of this paper to examine the adaptive significance of major genes, but it must be borne in mind that major genes are not a distinct class but are only one extreme of a frequency curve of gene size.

'Preadaptation' is used to mean the existence of a character which renders an organism potentially able to make use of a changed environment or to extend its original environmental limits.

Two other terms require definition: modifier and character. 'Modifier' is used in the sense of a gene which alters the expression of a major gene but which has no effect on its allele. A 'modifier complex' is thus an assemblage of minor genes, all of which affect the expression of the same major gene. The term 'character' has been used throughout this paper to mean a readily recognizable phenotypic effect or difference. Thus the large phenotypic effect associated with a major gene as compared with its allele would be a 'character', whereas the minute difference produced by a single minor gene would not be so described, though the cumulative action of a group of such genes would be said to control a 'character'.

Gene 'size' throughout this paper refers to the magnitude of the effect of a gene.

THE RELATIONSHIP BETWEEN GENE SIZE AND SELECTIVE VALUE

Consider the response of an organism to changing selective forces. It is a reasonable assumption that the variability present prior to the change in selective forces was effectively neutral. It by no means follows, however, that the gene differences present will all be of equal value under the new conditions. For instance, on the introduction of blackarm disease into a cotton-growing country, resistance genes previously of neutral effect become genes of very varying magnitudes. Areas where the crop already contains a proportion of plants carrying the strong resistance gene $\mathbf{B_2}$ will be 'sifted' by the selection pressure of the disease so that an increasing proportion of $\mathbf{B_2}$ plants will survive. This has, in fact, happened in many parts of India and Africa with the result that many of the Indian and African cottons carry a large proportion of $\mathbf{B_2}$ plants. In the areas climatically most suited to the spread of this disease the continued selection pressure of blackarm will give a premium to plants showing a greater resistance than $\mathbf{B_2}$ and so will be built up a modifier complex enhancing the strength of $\mathbf{B_2}$. Minor genes with additive effect *vis-à-vis* $\mathbf{B_2}$ will also be added until in the areas most subject to the disease something approaching immunity will be evolved.

At the other end of the scale will come areas in which the commercial cotton crop contains no plants carrying $\mathbf{B_2}$ or other strong resistance factors. The subjection of such an area to a series of yearly blackarm attacks may either eliminate the crop, as happened with the very susceptible Egyptian type crop in much of the U.S. cotton belt, or it will by its steady selection pressure favour plants carrying minor genes governing resistance. If a number of such genes exists in the crop there will be built up *slowly* a measure of resistance sufficient to control the disease under the conditions obtaining in that particular area. Minor gene resistance of this type is found in certain American Upland cottons—notably in Deltapine 14, Rowden B6 and to a lesser extent in some of the Stoneville types. Harland (1934) built up resistance of this type by constant selection in Sea Island, and although this resistance was not at first recognized by Knight & Clouston (1941) the presence of a weak resistance has since been confirmed by the writer.

Minor genes are not all of equal value—clearly there must be a gradation from the

minor type to the major type. Thus, in blackarm resistance, the genes $\mathbf{B_2}$ and $\mathbf{B_3}$ are both strong and each confers a high degree of resistance. The Asiatic gene $\mathbf{B_4}$ is slightly weaker than either $\mathbf{B_2}$ or $\mathbf{B_3}$ and the American Upland gene $\mathbf{B_1}$ is so weak that although its phenotype can be clearly recognized on some backgrounds it is lost on others (Knight, 1947). Other weak but identifiable resistance genes have been found in a *punctatum* cotton from Kufra oasis and in another *punctatum* of unknown origin. In addition, unidentifiable minor genes and modifiers also occur in blackarm resistance.

On the occurrence of preadaptation

In this consideration of the selective effect of a new disease it has been assumed that various resistance genes were already in existence although the crop had not previously been subjected to the disease. Experience has shown that preadaptation of this kind is not uncommon.

Such preadaptation is particularly well illustrated in the resistance of cotton (*Gossypium*) to blackarm disease. *Gossypium* is, in origin, a xerophytic or semi-xerophytic genus and *Bact. malvacearum* is a rain-borne disease. If, therefore, resistance has developed in 'response' to the selective effect of blackarm working in the rain areas in which cultivated cottons are grown, one would expect the wild species of *Gossypium* to be susceptible. An examination of these wild species,* however, shows *G. anomalum* to be almost immune. *G. somalense*, *G. klotzschianum* and its var. *davidsonii*, *G. armourianum*, *G. raimondii* and *G. aridum* are all resistant, whilst *G. harknessii* contains both resistant and susceptible types. *G. stocksii*, *G. sturtii*, *G. thurberi* and *G. tomentosum* are all susceptible.

An ecological examination of the wild *Gossypiums* shows that resistance to blackarm has not been developed under a selection pressure due to the presence of the disease. *G. anomalum*, which is almost immune to the disease, is found on the southern fringes of the Sahara across the width of Africa. The range of *G. somalense* overlaps that of *G. anomalum*, but the species is, if anything, rather more xerophytic. It, also, is resistant to blackarm. The New World species *G. klotzschianum*, *G. klotzschianum* var. *davidsonii*, *G. armourianum*, *G. raimondii*, *G. aridum* and *G. harknessii* are all from arid areas where they would not be subject to blackarm attack. Furthermore, there would be no survival of disease debris under such conditions, and seed infection would be impossible because of the length of time these wild seeds take to germinate. Since, by the very nature of these eco-climatic conditions, the possibility of the presence of blackarm disease is excluded, it is logical to suppose that the advent of this disease in the genus *Gossypium* was subsequent to the establishment of cotton as a cultivated crop in more mesophytic environments.

Further examples of preadaptation in *Gossypium* can be cited. Thus marked resistance to the virus disease Leaf Curl is found in some, but by no means in all, Sea Island cottons; it is also found in certain American Upland cottons from India and in some of the diploid *Gossypiums*, notably in *G. thurberi*, *G. armourianum*, *G. aridum* and *G. raimondii* from the New World and *G. stocksii* from Sind. Since Leaf Curl disease has been reported only from the Sudan and Nigeria, the resistance occurring in types of non-African origin is clearly preadaptational. Moreover, marked resistance to the Egyptian and Pink bollworms (*Earias insulana* and *Platyedra gossypiella*) has been found in *Gossypium thurberi* and *G. armourianum*. Both these bollworms are of Old World origin and their introduction

* In this connexion it should be noted that most of these wild *Gossypiums* had come from single plants.

into the New World was, in terms of evolution, very recent, so that the resistance shown by these two New World *Gossypiums* was not evolved under selection pressure from these pests.

A THEORY OF THE GENETIC NATURE OF PREADAPTATION

Where a character has been built up under selection, one of the following genetic situations will have arisen:

(1) A single gene, giving full expression to the character, established in the population.

(2) One or more major genes of lesser size established and intensified up to the level of full expression by the addition of cumulative minor genes and modifiers.

(3) An accumulation of minor genes all of small individual effect.

Obviously situation (1) will be established more quickly than number (2), provided a gene of the necessary magnitude exists. Similarly, situation (2) will be established more easily and quickly than (3) if any suitable major genes are present in the population. It follows that characters that are fully developed at their first appearance are most unlikely to depend on genetic situation (3) and may be expected in most cases to be governed by situation (1). On the other hand, adaptations developed slowly over a considerable period in response to selection pressure will, in general, be governed by a number of minor genes. In fact, the rate at which a character is established under selection may be used as an index of the probable size of the genes involved. *Preadaptational characters involving major differences will thus be typically controlled by one or a few large genes*, whereas adaptations which arise in 'response' to an existing selection pressure may be controlled either by major or minor genes or both, according to the size of the genes available.

Fisher (1930) considers that the range of selective intensity in which a factor is effectively neutral is very small. He concluded that the genes contributing to the genetic variance of a species must therefore be of small individual effect. Fisher was considering the situation in which a species is steadily modified by slow adaptive response to a slowly changing environment. Under man's selection, however, changes in selection pressure are often sudden and drastic and the conditions postulated by Fisher do not, therefore, hold. Such drastic environmental changes may greatly alter the magnitude of gene effects. Thus, to return to the blackarm example, genes conferring resistance are, so far as is known, of no effect in the absence of the disease and therefore fulfil Fisher's requirement for genes which will contribute to the variability of the species. As has been shown above, however, these genes, under the impact of blackarm, become genes of very varying and often very considerable effect.

Diseases tend to be of greater importance in the closely planted monocultures of modern crop production than in the sparser conditions more typical of wild growth. The advent of a new disease in a cultivated crop represents a sudden environmental change of the type under discussion and one might, therefore, expect to find preadaptation and, consequently, major gene control predominating in the genetics of disease resistance. A survey of recent plant-breeding literature shows the facts to be in accordance with this expectation, as is demonstrated by the list (in Appendix 1) of thirty-three crop plants in which major gene resistance to eighty-four pests and diseases has been demonstrated.

In view of this importance of major genes in the control of disease resistance, the writer made a survey of recent literature to ascertain whether major genes are of equal

importance in the control of other economic characters. The results of this survey, tabulated in Appendix 2, show that in thirty-eight plants some 160 economic characters have so far been found to be wholly or partly under major gene control. These economic characters can be divided into four classes according to the evolutionary road by which major gene control became established.

(1) Those characters valued by man which have no selective value, either positive or negative, in the wild state. Such characters are preadaptational and therefore their control by one or a few major genes is not unexpected.

(2) Those characters which would have negative selection value in the wild species but which are valued by man. The survival of major genes governing characters of this type can reasonably be attributed to man having noticed the occasional rare mutation and propagated it for his own ends.

(3) Certain characters which by their very nature must be operated by a switch gene mechanism. These are the characters in which no intermediate stage between presence and absence is possible so that a slow building up of minor genes is out of the question, although they can be added after the main gene has brought the trait into being.

(4) Characters in which all degrees of expression are of value in appropriate environments. Such characters are usually manifested in the wild state as a cline developed in response to a continuously varying environmental factor, such as rainfall. The genetic control of such a cline would typically be multifactorial and it might be supposed that the individual genes would be small in effect. In actual fact, however, investigation of such characters in the genetically more uniform populations studied by the plant breeder has frequently shown that some of the genes involved are of large effect.

The application of this grouping to the characters listed in Appendix 2 shows that 49% of them belong to group (1), 28% to group (4), 19% to group (2), and only 4% to group (3). Although this grouping is arbitrary in certain cases, this fault is not likely materially to affect the totals in the four groups, and it is interesting to note the preponderance of group (1) traits—the preadaptational characters. It is also interesting to note the relative unimportance of group (3). It thus seems probable that preadaptation has played an important role not only in the evolution of disease resistance but also in the evolution of economic characters in general.

Discussion

It is clear from the lists in Appendices 1 and 2 that major genes are more common in the control of economic characters than current genetic ideas would lead one to suppose. It is not suggested that control by a single powerful major gene unaccompanied by minor genes is likely to be a common genetic situation in many economic characters. Genes which by themselves give complete expression to a character appear to be rare; hence in economic characters, man will have added, by constant selection towards the maximum expression, a complex of minor genes. Thus, particularly where a character would have been of value in the wild state, a common form of genetic control is likely to be by one or two large genes accompanied by a number of minor genes and modifiers. This type of control will be particularly common where both alleles governing a character have been of value under different environmental conditions in the wild state.

Even if a character is basically controlled by major genes it may yet be too complex to be analysed genetically. This applies to compound 'characters' which are the result

of interaction of several characters, and hence it applies to many of the so-called 'quantitative' characters. Thus, for example, 'ginning out-turn'* in cotton is of great economic importance. One would not, however, expect it to show 'simple' inheritance because ginning out-turn is dependent on (1) density of population of lint hairs on the seed coat, (2) length of the lint hairs, (3) weight of the lint hairs per unit length (this, itself, is dependent on (*a*) degree of thickening of the hair and (*b*) hair diameter), and (4) size of seed. Furthermore, ginning out-turn has been of value to man ever since cotton became a commercial crop, so that over and above any larger differences due to major genes, one would expect man's selection to have built up a considerable complex of minor and modifying genes. Where two cotton types share several, but not all, of the characters which together determine ginning out-turn, then, on intercrossing them, one might expect to obtain some semblance of a Mendelian ratio. This probably explains the bimodal curves obtained by O'Kelly & Hull (1930) in some of their ginning out-turn inheritance studies, in which they found that 'where segregation was sufficiently clear-cut to give definite indications, it appeared that lint percentage was controlled by a single factor pair'. They also noted that 'in crosses between species and crosses between upland strains where the percentage differences were narrow, it was not possible to determine definitely the nature of the segregation'. This is in accord with the theory given above: in the interspecies crosses there would have been too many variables for major gene segregation to be evident, whereas in the upland crosses involving small percentage differences, the major genes were presumably the same in each parent and the differences would have been due to modifiers and/or minor genes.

By their very nature, many valuable crop characters are likely to have complex inheritance, but such 'blending' inheritance is not *necessarily* entirely polygenic. For breeding purposes an attempt should be made to reduce complex characters to their integral components in the hope that these can be examined separately. Even in the more complex characters there is still reasonable hope of finding key genes governing inheritance, provided the complexity can be reduced to its component interacting characters.

In the field of disease resistance, plant breeders might with benefit consider the implications of preadaptation. Often the local crop carries no major genes governing resistance to a particular disease. For example, major genes governing resistance to leaf curl virus do not appear to be present in Sakel cotton. Thus the NT 2 variety of Sakel was originally fully susceptible to this virus disease. By constant selection, however, it was rendered more and more resistant until the later substrains approached immunity. S. H. Evelyn's (unpublished) work on breeding for leaf curl resistance in Domains Sakel cotton, again illustrates the synthesis of polygenic resistance to the disease. On the other hand, Sea Island and other imported types grown in the Sudan have differed markedly in their leaf curl susceptibility, some being highly susceptible and others almost immune. Since leaf curl is unknown outside Africa this resistance is presumably preadaptational. Such types might thus be expected to provide a useful source of major resistance genes for future work. Furthermore, a major gene governing a preadaptational character is unlikely to be accompanied by any minor gene or modifier complex so that it should not normally lose appreciably in strength when transferred to other varieties and species.

To the plant breeder engaged in hybridization, major genes are of greater value than minor genes because they are far more easily handled. Modern genetics tends to stress

* The percentage of cotton lint produced by a given weight of seed cotton.

the minor gene outlook, but any overstressing of this is likely to be to the detriment of applied genetics. Breeders who have followed the minor gene school of thought might be disinclined to examine their material in sufficient detail. Plant breeders are often content with the so-called 'practical' outlook, whereby their immediate problem is settled without any genetical information being acquired which would help others to deal with similar problems. If a more wholehearted attempt at genetical analysis were made as a routine practice, it is possible that many more cases of major gene inheritance of economic characters would be discovered. In intraspecific hybridization, two or three major genes each showing partial dominance, even if unaccompanied by minor genes and modifying factors, will often present all the appearances of polygenic inheritance. In interspecific hybridization, characters not infrequently show blending inheritance in F_2 yet segregate clearly in backcross generations after the genetic background of one or other parental species has been re-established in the hybrid stock. In the absence of detailed genetical analysis there is considerable danger of misinterpretation of such situations in terms of minor genes.

Summary

It is considered that preadaptation is not uncommon in economic characters. Notable examples are the resistance of many wild xerophytic species of *Gossypium* to the rain-borne disease *Bact. malvacearum*, the resistance of several cottons of non-African origin to the virus disease leaf curl (a disease unknown outside the Sudan and Nigeria), and the marked resistance of certain New World species to the Egyptian and Pink bollworms.

It is argued that preadaptational characters involving major differences will typically be found to be controlled by one or a few large genes, whereas adaptations which arise in 'response' to an existing selection pressure may be controlled either by major or minor genes, or both, according to the strength of the genes available.

Preadaptation is thought to have played a major part in the evolution of disease and pest resistance and to this is attributed the predominance of major gene control in resistance. In this connexion a list is given of thirty-three crop plants in which major gene resistance to eighty-four pests and diseases has been demonstrated.

Major gene control is shown to be of considerable importance in a large number of economic characters other than disease resistance and a list is given of thirty-eight plants in which 160 economic characters have been found to be wholly or partly under major gene control. Approximately 50% of these characters are classified as preadaptational. It is suggested that major genes are more common in the control of economic characters than is generally supposed. Control by a single powerful major gene, unaccompanied by minor genes, is unlikely to be common. Single genes alone rarely give complete expression to a character, so that man's constant selection towards the maximum expression of each economic character will have added a complex of minor and modifying genes to the original strong gene.

By their very nature, many valuable crop characters are likely to have complex inheritance, but such 'blending' inheritance is not necessarily entirely polygenic. For breeding purposes an attempt should be made to reduce complex characters to their integral components to facilitate genetic analysis.

It is a pleasure to record my gratitude to Mr J. B. Hutchinson for his constructive criticisms and helpful discussions.

APPENDIX 1

Diseases and pests, resistance to which is controlled, at least in part, by major genes

Wheat

Puccinia graminis tritici (Melchers & Parker, 1922)
P. glumarum (Biffen, 1905)
P. triticina (Waterhouse, 1930)
P. rubigo-vera tritici (Mains, 1934)
Tilletia tritici (Briggs, 1931)
T. levis (Gaines & Smith, 1933)
Ustilago tritici (Tingey, 1934)
Urocystis tritici (Shen, 1934)
Erysiphe graminis (Waterhouse, 1930)
Mosaic (yellow) (Miyake, 1938)
Mosaic (green) (Miyake, 1938)
Chlorops pumilionis (Jasnowski, 1938)
Cephus cinctus (Putnam, 1942)

Barley

Puccinia graminis tritici (Powers & Hines, 1933)
P. simplex (Straib, 1937)
Ustilago nuda (Nahmmacher, 1931)
Erysiphe graminis hordei (Dietz & Murphy, 1930*a*)
Helminthosporium (Kuckuck, 1930)

Oats

Puccinia coronata avenae (Dietz & Murphy, 1930*b*)
P. graminis avenae (Waterhouse, 1930)
Ustilago avenae (Reed, 1932)
U. levis (Reed, 1932)

Rice

Helminthosporium oryzae (Nagai & Hara, 1930)
Piricularia oryzae (Ramiah & Ramaswami, 1936)
Cercospora oryzae (Nakamori, 1936)

Setaria

Ustilago crameri (Yu, 1942)

Sorghum

Sphacelotheca cruenta (Marcy, 1937)
S. sorghi (Marcy, 1937)
Pythium root rot (Bowman, Martin, Melchers & Parker, 1937)
(?) Chinch Bug (Dahms, 1943)

Maize

Puccinia sorghi (Mains, 1931)
Gibberella saubinetii (where due to non-sugary gene) (Senn, 1932)
Bact. stewartii (Wellhausen, 1937)
(?) *Ustilago zeae* (Saboe & Hayes, 1941)
Helminthosporium maydis (Ullstrup, 1941)

Cotton

Bact. malvacearum (Knight & Clouston, 1939)
Jassid (Knight, unpublished)

Potato

Synchytrium endobioticum (Salaman & Lesley, 1923)
Phytophthora infestans (Black, 1945)
Actinomyces scabies (Krantz & Eide, 1941)
Virus A (Cockerham, 1943)
Virus B (Cockerham, 1943)
Virus C (Cockerham, 1943)
Virus X (Cockerham, 1943)

Apple

(?) Apple cedar rust (Moore, 1940)

Capsicum

Tobacco mosaic virus (Holmes, 1937)

Sunflower

Homoesoma nebulella (Plaček, 1936)
Orobanche (Plaček, 1936)

Tobacco

Tobacco-mosaic (Holmes, 1938)

Gram (Cicer)

Fusarium wilt (Ayyar & Iyer, 1936)

Pea (Pisum)

Fusarium orthoceras var. *pisi* (Wade, Zaumeyer & Harter, 1938)
F. oxysporum f. pisi race 2 (Walker, Delwiche & Hare, 1944)
Mildew (Harland, 1946)

Cowpea (Vigna sinense)

Powdery mildew (Dundas, 1939)

Beans (Vigna sesquipedalis)

Powdery mildew (Dundas, 1939)

Beans (Phaseolus)

Colletotrichum Lindemuthianum (Burkholder, 1918)
Uromyces appendiculatus (Wingard, 1933)
Mosaic (Pierce & Walker, 1933)
Alfalfa mosaic (Wade & Zaumeyer, 1940)
Erysiphe polygoni (Dundas, 1939)
Heterodera marioni (Barrons, 1940)

Soya bean

Empoasca fabae (Johnson & Hollowell, 1935)

Tomato

Cladosporium fulvum (Guba, 1936)
Fusarium wilt (Bohn & Tucker, 1940)
Septoria lycopersici (Andrus & Reynard, 1945)
Alternaria solani (Reynard & Andrus, 1945)

Ground nuts

Leaf-spot fungus (1) (Higgins, 1938)
Leaf-spot fungus (2) (Higgins, 1938)

Onion

Colletotrichum circinans (Rieman, 1930, 1931)

Cabbage

Fusarium conglutinans (Walker, 1930)

Brussels sprouts

Fusarium (Blank & Walker, 1933)

Kohlrabi

Fusarium (Blank & Walker, 1933)

Flax

Melampsora lini (Henry, 1930)
Colletotrichum lini (Houston & Stanford, 1945)

Beet

Curly-top (Owen & Abegg, 1938)

Cucumber

Cladosporium cucumerinum (Bailey, 1939)
Mosaic (Shifriss, Myers & Chupp, 1942)

Lettuce

Bremia lactucae (Jagger & Whitaker, 1940)
Erysiphe cichoracearum (Whitaker & Pryor, 1941)
Brown Blight (Jagger, 1940)

Water melon

Colletotrichum lagenarium (Poole, 1944)

Melon

Erysiphe cichoracearum (Jagger, Whitaker & Porter, 1938)

Cajanus

(?) Wilt (Shaw, 1936)

Grape

Phylloxera vitifoliae (Richm, 1940)
(?) *Peronospora viticola* (Husfeld, 1930)

APPENDIX 2

Economic characters which are controlled, at least in part, by major genes

Group*	Character
	Wheat
4	Awn length (see Sansome, 1939)
2	Awns, absence of (see Sansome, 1939)
1	Ear density and shape (see Sansome, 1939)
1	Grain colour (see Sansome, 1939)
2	Non-shattering (Miczynski, 1930)
1	Brittleness (Raum, 1931)
1	Waxy endosperm (Watkins & Cory, 1931)
4	Earliness (Crescini, 1933)
3	Spring versus winter habit (see Sansome, 1939)
4	Grain weight (Jasnowski, 1935)
4	Grains per spikelet (Jasnowski, 1935)
4	Spikelets per ear (Jasnowski, 1935)
1	Absence of sprouting in ear (Åkerman, 1936)
1	(?) Gluten quality (Worzella, 1934)
1	Hollow versus solid stem (Platt, Darroch & Kemp, 1941)

* See p. 374.

Group	Character
	Barley
4	Rows per ear (Huber, 1931)
1	Hulled versus naked (Huber, 1931)
1	Grain colour (see Sansome, 1939)
2	Absence of dormancy (Moormann, 1942)
4	Earliness (Barbacki, 1930)
1	Starchy versus glutinous (Kasiwada, 1930)
4	Plant height (Swenson & Wells, 1944)
4	Rachis internode number (Swenson & Wells, 1944)
2	Non-barbed awns (Huber, 1931)
1	Ear density (see Sansome, 1939)
1	Ear length (Huber, 1931)
1	Ear shape (Huber, 1931)
1	Branched ears (Huber, 1931)
3	Spring versus winter habit (see Sansome, 1939)
2	Brittle awns (Tavčar, 1939)
2	Non-brittle rachis (Johnson & Åberg, 1943)
2	Awnless (see Sansome, 1939)
	Oats
2	Awnless (Florell, 1931)
1	Hulled versus hull-less (Love & McRostie, 1919)
2	Absence of dormancy (Moormann, 1942)
4	Earliness (Shaw & Bose, 1933)
1	Grain colour (see Sansome, 1939)
	Rice
2	Awnless (Jones, 1933)
4	Awn length (Mitra & Ganguli, 1932)
4	Earliness (Jones, 1933)
1	Glume length (Jones, 1933)
1	Compact versus spreading habit (Ramiah, 1930)
1	Grain colour (Ramiah, 1930)
1	Glutinous versus starchy (Ramiah, Jobitharaj & Mudaliar, 1931)
1	Long versus short kernels (Ramiah *et al.* 1931)
1	Lax versus dense panicle (Ramiah *et al.* 1931)
1	Brittleness (Jones, 1933)
2	Non-shattering (Jones, 1933)
4	Erect, or prostrate tillers (Ting, 1933)
4	Early short type (Miyazawa, 1934)
1	Lodging versus non-lodging (Ramiah & Dharmalingam, 1934)
1	Perfume (Kadam & Patankar, 1938)
4	Floating habit (Ramiah & Ramaswami, 1941)
	Sorghum
4	Normal versus dwarf (early) (Sieglinger, 1932)
4	Tall versus normal (Karper, 1932)
1	Pearly versus chalky grain (Ayyangar, Vijiaraghavan, Ayyar & Rao, 1934)
1	Waxy endosperm (Karper, 1933)
1	Awns versus awnless (Sieglinger, Swanson & Martin, 1934)
1	Pithy versus juicy stalks (Ayyangar, Ayyar, Rao & Nambiar, 1936)
1	Insipid versus sweet stalks (Ayyangar *et al.* 1936)
4	Short early versus tall late (Ayyangar, Ayyar & Nambiar, 1937)
1	Twin seeded versus single seeded spikelets (Stephens & Quinby, 1938)

Group	Character
2	Cleistogamy (Ayyangar & Ponnaiya, 1939*b*)
1	(?) Absence of cyanogenetic glucosides (Franzke, Puhr & Hume, 1939)
4	Time of floral initiation and maturity date (Quinby & Karper, 1945)
	Sudan grass (Sorghum Sudanense)
4	Tillers versus tillerless (Ayyangar & Ponnaiya, 1939*a*)
4	Tillers all flowering together versus un-uniform flowering (Ayyangar & Ponnaiya, 1939*a*)
4	Long versus short awn (Ayyangar & Ponnaiya, 1939*a*)
	Maize
1	Endosperm colour (see Hayes & Immer, 1942)
1	Aleurone colour (see Hayes & Immer, 1942)
1	Endosperm nature (see Hayes & Immer, 1942)
1	Pericarp and cob colour (see Hayes & Immer, 1942)
1	Vitamin A content (Hauge & Trost, 1930)
1	(?) Nicotinic acid (Mather & Barton-Wright, 1946)
1	Rows per cob (Tavčar, 1935)
	Rye
1	Brittleness (Lada, 1933)
	Pennisetum
4	Bristly versus non-bristly (Ayyangar & Hariharan, 1936)
1	Sugary versus starchy grains (Patel, 1941)
	Setaria
4	Bristle length (Ayyangar, Narayanan & Rao, 1933)
1	Dense versus lax (Ayyangar & Sarma, 1933)
	Panicum
1	Grain colour (Ayyangar & Rao, 1938)
	Cotton
1	Long versus short sympodia (Kearney, 1930)
1	Normal versus 'cluster' (Thadani, 1923)
4	Degree of boll dehiscence (Abraham, 1934)
1	Lint colour (Harland, 1939)
1	Lint percentage (O'Kelly & Hull, 1930)
1	Presence or absence of lint (Afzal & Hutchinson, 1933)
1	'Expansive' lint (Hutchinson, Silow & Stephens, 1947)
4	5-loc boll (Anonymous, 1944–5)
	Capsicum
2	Absence of pungency (Deshpande, 1935)
4	(?) Number of locules (Miller & Fineman, 1938)
	Tobacco
1	Nicotine content (Hackbarth & Sengbusch, 1935)
4	Incompatibility and fertility (East, 1932)
	Blackberry
2	Thornlessness (Stene & Odland, 1938)
	Sweet clover (Melilotus)
1	Coumarin content (Stevenson & White, 1940)
	White clover (Trifolium)
1	Absence of cyanogenetic glucoside (Corkill, 1942)

Group	Character
	Raspberry
1	Fruit colour (Crane & Lawrence, 1931)
	Coconut
1	Makapuno versus normal (Torres, 1937)
	Peach
1	Melting flesh (Bailey & French, 1932)
1	Clingstone versus freestone (Bailey & French, 1932)
	Grape
1	Early branching (Stummer & Frimmel, 1932)
1	Colour of juice (Branas, Bernon & Levadoux, 1938)
	Pisum
4	Height (Mendel)
2	Thickness of pod-wall (Nilsson, 1932)
	Beans (Vicia)
4	Number of seeds per pod (Sirks, 1931)
4	Internode number (Sirks, 1931)
4	Stem length (Sirks, 1931)
	Beans (Phaseolus)
2	'Stringless' pod (Prakken, 1934)
4	Climbing versus bush habit (Schreiber, 1932)
2	Absence of parchment in pod wall (Prakken, 1934)
	Soya bean
1	Seed coat colour (Woodworth, 1932)
1	Pubescence (Woodworth, 1932)
4	Lateness associated with tall habit (Woodworth, 1932)
4	Seeds per pod (Takahashi, 1934)
	Tomato
4	Tall versus dwarf (MacArthur, 1931)
1	Smooth versus peach skin (MacArthur, 1931)
1	Fruit colour (MacArthur, 1931)
4	Internode length (MacArthur, 1931)
1	Self-pruning habit (MacArthur, 1931)
1	Smooth versus fasciated fruit (MacArthur, 1931)
4	Uniform ripening (Anonymous, 1942)
2	Determinate versus indeterminate habit (Currence, 1932)
4	Number of locules (Yeager, 1937)
1	Fruit shape (Yeager, 1937)
	Ground nuts
4	Late versus early (Patel, John & Seshadri, 1936)
1	Long versus short seed (Hull, 1937)
4	Growth habit (Valencia—Spanish-Runner) (Hull, 1937)
	Carrot
1	Root shape (Frimmel & Lauche, 1938)
	Sesamum
4	Branching (Nohara, 1933)
4	Extra pods in leaf axil (Langham, 1945)
1	Calcium oxalate in seed (Nohara, 1933)

Group	Character
	Beet
3	Annual versus biennial (Munerati, 1931)
2	Self-fertility (Owen, 1938)
	Strawberry
1	Red versus white fruit (Fedorova, 1935)
2	Ever-bearing versus normal (Fedorova, 1935)
	Lettuce
1	Leaf lobing (Durst, 1930)
2	Non-prickly midribs (Durst, 1930)
3	Photoperiodism (Bremer & Grana, 1935)
3	Heading versus rosetting (Bremer & Grana, 1935)
	Cucumber (Cucumis sativus)
2	Fine spines versus coarse (Poole, 1944)
2	Few versus numerous spines (Poole, 1944)
2	Absence of spines (Poole, 1944)
2	Determinate versus indeterminate growth (Poole, 1944)
1	Fruit colour (Poole, 1944)
4	Tall versus short (Poole, 1944)
2	Tender skin (Poole, 1944)
1	Smooth versus rough fruit (Poole, 1944)
	Cucurbita pepo
1	Fruit shape (Sinnott & Hammond, 1930)
	Watermelon (Citrullus vulgaris)
1	Smooth versus furrowed fruit (Poole, 1944)
4	Seed weight (Weetman, 1937)
1	Elongate versus spherical fruit (Weetman, 1937)
1	Flesh colour (Porter, 1937)
2	Non-explosive rind (Poole, 1944)
2	Tender rind (Porter, 1937)
	Celery
1	Pithy versus non-pithy (Jones, 1932; Emsweller, 1932)
	Lupins
2	Alkaloid-free (Hackbarth & Sengbusch, 1934)
1	Soft versus hard skin (Sengbusch, 1938)
2	Non-splitting pods (Hackbarth, 1938)
1	White 'grain' (Hackbarth, 1938)
	Yeast
3	Ability to ferment sugar (Spiegelmann, Lindegren & Lindegren, 1945)

REFERENCES

ABRAHAM, P. (1934). *Proc. Assoc. Econ. Biol. Coimbatore*, **2**, 22.

AFZAL, M. & HUTCHINSON, J. B. (1933). *Indian J. Agric. Sci.* **3**, 1124.

ÅKERMAN, Å. (1936). *Züchter*, **8**, 25–9.

ANDRUS, C. F. & REYNARD, G. B. (1945). *Phytopathology*, **35**, 16–24.

ANONYMOUS (1944–5). *Prog. Rep. Cott. Genet. Res. Scheme, Indore, C.I.* 1944–5. (Mimeograph.)

ANONYMOUS (1942). *Bull. Mass. Agric. Exp. Sta.* no. 338.

AYYANGAR, G. N. R. & HARIHARAN, P. V. (1936). *Madras Agric. J.* **24**, 235–7.

AYYANGAR, G. N. R. & PONNAIYA, B. W. X. (1939*a*). *Proc. Indian Acad. Sci.* **10**, B, 237–54.
AYYANGAR, G. N. R. & PONNAIYA, B. W. X. (1939*b*). *Curr. Sci.* **8**, 418–19.
AYYANGAR, G. N. R. & RAO, P. K. (1938). *Madras Agric. J.* **26**, 195–206.
AYYANGAR, G. N. R. & SARMA, P. S. (1933). *Indian J. Agric. Sci.* **3**, 557–8.
AYYANGAR, G. N. R., AYYAR, M. A. S. & NAMBIAR, A. K. (1937). *Madras Agric. J.* **25**, 107–18.
AYYANGAR, G. N. R., NARAYANAN, T. R. & RAO, T. N. (1933). *Indian J. Agric. Sci.* **3**, 207–18.
AYYANGAR, G. N. R., AYYAR, M. A. S., RAO, V. P. & NAMBIAR, A. K. (1936). *Madras Agric. J.* **24**, 247–8.
AYYANGAR, G. N. R., VIJIARAGHAVAN, G., AYYAR, M. A. S. & RAO, V. P. (1934). *Indian J. Agric. Sci.* **4**, 96–9.
AYYAR, R. V. & IYER, B. R. (1936). *Proc. Indian Acad. Sci.* **3**, B, 438–43.
BAILEY, J. S. & FRENCH, A. P. (1932). *Proc. Amer. Soc. Hort. Sci.* **29**, 127–30.
BAILEY, R. M. (1939). *Proc. Amer. Soc. Hort. Sci.* **36** (1938), 645–6.
BARBACKI, S. (1930). *Mém. Inst. polon Econ. rur.* **11**, 579–610.
BARRONS, K. C. (1940). *J. Hered.* **31**, 35–8.
BIFFEN, R. H. (1905). *J. Agric. Sci.* **1**, 4–48.
BLACK, W. (1945). *Ann. Appl. Biol.* **32**, 279–80.
BLANK, L. M. & WALKER, J. C. (1933). *J. Agric. Res.* **46**, 1015–22.
BOHN, G. W. & TUCKER, C. M. (1940). *Research Bull. Mo. Agric. Exp. Sta.* **311**, 1–82.
BOWMAN, D. H., MARTIN, J. H., MELCHERS, L. E. & PARKER, J. H. (1937). *J. Agric. Res.* **55**, 105–15.
BRANAS, J., BERNON, G. & LEVADOUX, L. (1938). *Plant Breed. Abstr.* **10**, 308.
BREMER, A. H. & GRANA, J. (1935). *Plant Breed. Abstr.* **6**, 90–1.
BRIGGS, F. N. (1931). *J. Agric. Res.* **42**, 307–13.
BURKHOLDER, W. H. (1918). *Phytopathology*, **8**, 353–9.
COCKERHAM, G. (1943). *Ann. Appl. Biol.* **30**, 105–8.
CORKILL, L. (1942). *N.Z. J. Sci. Tech.* **23**, 178B–193B.
CRANE, M. B. & LAWRENCE, W. J. C. (1931). *J. Genet.* **24**, 243–55.
CRESCINI, F. (1933). *Ann. Tec. Agr., Roma*, **6**, 36–56.
CURRENCE, T. M. (1932). *Proc. 6th Int. Congr. Genet. N.Y.* **2**, 32–4 (Abstr.).
DAHMS, R. G. (1943). *J. Amer. Soc. Agron.* **35**, 704–15.
DESHPANDE, R. B. (1935). *Indian J. Agric. Sci.* **5**, 513–16.
DIETZ, S. M. & MURPHY, H. C. (1930*a*). *Phytopathology* (Abstr.), **20**, 119–20.
DIETZ, S. M. & MURPHY, H. C. (1930*b*). *Phytopathology* (Abstr.), **20**, 120.
DUNDAS, B. (1939). *Phytopathology*, **29**, 824.
DURST, C. E. (1930). *Bull. Ill. Agric. Exp. Sta.* **356**, 239–341.
EAST, E. M. (1932). *Genetics*, **17**, 175–202.
EMSWELLER, S. L. (1932). *Proc. Amer. Soc. Hort. Sci.* **29**, 480–5.
FEDOROVA, N. JA. (1935). *Plant Breed. Abstr.* **6**, 434.
FISHER, R. A. (1930). *The Genetical Theory of Natural Selection.* Oxford: Clarendon Press.
FLORELL, V. H. (1931). *J. Agric. Res.* **43**, 365–86.
FRANZKE, C. J., PUHR, L. F. & HUME, A. N. (1939). *Tech. Bull. S. Dak. St. Coll.* no. 1, pp. 1–51.
FRIMMEL, F. & LAUCHE, K. (1938). *Plant Breed. Abstr.* **9**, 127–8.
GAINES, E. F. & SMITH, W. K. (1933). *J. Amer. Soc. Agron.* **25**, 273–84.
GUBA, E. F. (1936). *Phytopathology*, **26**, 382–6.
HACKBARTH, J. (1938). *Plant Breed. Abstr.* **9**, 76.
HACKBARTH, J. & SENGBUSCH, R. VON (1934). *Plant Breed. Abstr.* **5**, 234.
HACKBARTH, J. & SENGBUSCH, R. VON (1935). *Plant Breed. Abstr.* **5**, 247.
HARLAND, S. C. (1934). *Emp. Cott. Gr. Rev.* **11**, 301.
HARLAND, S. C. (1939). *The Genetics of Cotton.* London: Jonathan Cape.
HARLAND, S. C. (1946). Reported in *Nature, Lond.*, **157**, 36.
HAUGE, S. M. & TROST, J. F. (1930). *J. Biol. Chem.* **86**, 167–72.
HAYES, H. K. & IMMER, F. R. (1942). *Methods of Plant Breeding.* New York and London: MacGraw-Hill Book Co.
HENRY, A. W. (1930). *Phytopathology*, **20**, 707–21.
HIGGINS, B. B. (1938). *Proc. 39th Ann. Convent. Ass. S. Agric. Wkrs., Atlanta, Ga.* pp. 57–8 (Abstr.).

HOLMES, F. O. (1937). *Phytopathology*, **27**, 637–42.
HOLMES, F. O. (1938). *Phytopathology*, **28**, 553–61.
HOUSTON, B. R. & STANFORD, B. H. (1945). *Plant Dis. Rep.* **29**, 572–3.
HUBER, J. A. (1931). *Z. Zücht.* A, **16**, 394–464.
HULL, F. H. (1937). *Tech. Bull. Fla. Agric. Exp. Sta.* no. 314, 46 pp.
HUSFELD, B. (1930). *Plant Breed. Abstr.* **2**, 93.
HUTCHINSON, J. B., SILOW, R. A. & STEPHENS, S. G. (1947). *The Evolution of 'Gossypium'*. Oxford University Press.
JAGGER, I. C. (1940). *Phytopathology*, **30**, 53–64.
JAGGER, I. C. & WHITAKER, T. W. (1940). *Phytopathology*, **30**, 427–33.
JAGGER, I. C., WHITAKER, T. W. & PORTER, D. R. (1938). *Phytopathology*, **28**, 671.
JASNOWSKI, S. (1935). *Plant Breed. Abstr.* **6**, 263.
JASNOWSKI, S. (1938). *Arch. Nauk. Biol.* **8**, Fasc. **3**, pp. 1–60.
JOHNSON, H. W. & HOLLOWELL, E. A. (1935). *J. Agric. Res.* **51**, 371–81.
JOHNSON, I. J. & ÅBERG, E. (1943). *J. Amer. Soc. Agron.* **35**, 101–6.
JONES, H. A. (1932). *Proc. Amer. Soc. Hort. Sci.* **29**, 572–81.
JONES, J. W. (1933). *J. Agric. Res.* **47**, 771–82.
KADAM, B. S. & PATANKAR, V. K. (1938). *Chron. Bot.* **4**, 496–7.
KARPER, R. E. (1932). *Amer. Nat.* **66**, 511–29.
KARPER, R. E. (1933). *J. Hered.* **24**, 257–62.
KASIWADA, S. (1930). *Plant Breed. Abstr.* **1**, no. 4, 24.
KEARNEY, T. H. (1930). *J. Agric. Res.* **41**, 349.
KNIGHT, R. L. (1944). *J. Genet.* **46**, 1–27.
KNIGHT, R. L. (1946). *Emp. J. Exp. Agric.* **14**, 153–74.
KNIGHT, R. L. (1947). *J. Genet.* **48**, 43–50.
KNIGHT, R. L. & CLOUSTON, T. W. (1939). *J. Genet.* **38**, 133–59.
KNIGHT, R. L. & CLOUSTON, T. W. (1941). *J. Genet.* **41**, 391–409.
KRANTZ, F. A. & EIDE, C. J. (1941). *J. Agric. Res.* **63**, 219–31.
KUCKUCK, H. (1930). *Züchter*, **2**, 68–72.
LADA, P. (1933). *Plant Breed. Abstr.* **5**, 118.
LANGHAM, D. G. (1945). *J. Hered.* **36**, 245–53.
LOVE, H. H. & MCROSTIE, G. P. (1919). *Amer. Nat.* **53**, 5–32.
LYSENKO, T. D. (1946). *Heredity and its Variability*. New York: King's Crown Press.
MACARTHUR, J. W. (1931). *Trans. Roy. Soc. Can.* **18**, 1–19.
MAINS, E. B. (1931). *J. Agric. Res.* **43**, 419–30.
MAINS, E. B. (1934). *Phytopathology*, **24**, 1257–61.
MARCY, D. E. (1937). *Plant Breed. Abstr.* **7**, Abstr. 1261, p. 400.
MATHER, K. (1943*a*). *Nature, Lond.*, **151**, 68–71.
MATHER, K. (1943*b*). *Biol. Rev.* **18**, 32–64.
MATHER, K. & BARTON-WRIGHT, E. C. (1946). *Nature, Lond.*, **157**, 109–10.
MELCHERS, L. E. & PARKER, J. H. (1922). *Phytopathology* (Abstr.), **12**, 31–2.
MICZYNSKI, K. (1930). *Polish Agric. and Forests. Ann.* **23**, 1–36.
MILLER, J. C. & FINEMAN, Z. M. (1938). *Proc. Amer. Soc. Hort. Sci.* (1937), **35**, 544–50.
MITRA, S. K. & GANGULI, P. M. (1932). *Indian J. Agric. Sci.* **2**, 271–9.
MIYAKE, M. (1938). *Jap. J. Genet.* **14**, 239–42.
MIYAZAWA, B. (1934). *Proc. Crop. Sci. Soc. Japan*, **6**, 156–8.
MOORE, R. C. (1940). *Proc. Amer. Soc. Hort. Sci.* (1939), 1940, **37**, 242–4.
MOORMANN, B. (1942). *Plant Breed. Abstr.* **15**, 35.
MUNERATI, O. (1931). *Plant Breed. Abstr.* **2**, 78.
NAGAI, I. & HARA, S. (1930). *Jap. J. Genet.* **5**, 140–4.
NAHMMACHER, J. (1931). *Phytopath. Z.* **4**, 597–630.
NAKAMORI, E. (1936). *Pl. Breed. News*, pp. 823–34.
NILSSON, E. (1932). *Plant Breed. Abstr.* **2**, 215.
NOHARA, S. (1933). *J. Coll. Agric. Tokyo*, **12**, 228–386.

O'Kelly, J. F. & Hull, W. W. (1930). *Tech. Bull. Miss. Agric. Exp. Sta.* **18**.
Owen, F. V. (1938). *Plant Breed. Abstr.* **9**, 93.
Owen, F. V. & Abegg, F. A. (1938). *Proc. Amer. Soc. Sug. Beet. Tech.* p. 72 (Abstr.).
Patel, J. S., John, C. M. & Seshadri, C. R. (1936). *Proc. Indian Acad. Sci.* **3**, B, 214–33.
Patel, Z. H. (1941). *Proc. 28th Indian Sci. Congr. Benares*, Pt III, Sect. Agric. Abstr. **40**, 258.
Pierce, W. H. & Walker, J. C. (1933). *Canner*, **77**, 7–9.
Plaček, E. M. (1936). *Plant Breed. Abstr.* **7**, 322–3.
Platt, A. W., Darroch, J. G. & Kemp, H. J. (1941). *Sci. Agric.* **22**, 216–24.
Poole, C. F. (1944). *J. Hered.* **35**, 122–8.
Porter, D. R. (1937). *Hilgardia*, **10**, 489–509.
Powers, L. & Hines, L. (1933). *J. Agric. Res.* **46**, 1121–9.
Prakken, R. (1934). *Genetica*, **16**, 177–296.
Putnam, L. G. (1942). *Sci. Agric.* **22**, 594–607.
Quinby, J. R. & Karper, R. E. (1945). *J. Amer. Soc. Agron.* **37**, 916–36.
Ramiah, K. (1930). *Mem. Dep. Agric. India. Bot.* **18**, 211–27.
Ramiah, K. & Dharmalingam, S. (1934). *Indian J. Agric. Sci.* **4**, 880–94.
Ramiah, K. & Ramaswami, K. (1936). *Proc. Ind. Acad. Sci.* **3**, B, 450–8.
Ramiah, K. & Ramaswami, K. (1941). *Indian J. Agric. Sci.* **11**, 1–8.
Ramiah, K., Jobitharaj, S. & Mudaliar, S. D. (1931). *Mem. Dep. Agric. India. Bot.* **18**, 229–59.
Raum, H. (1931). *Z. Zücht. A.* **16**, 161–254.
Reed, G. M. (1932). *Proc. 6th Int. Cong. Genet. N.Y.* **2**, 164–5.
Reynard, G. B. & Andrus, C. F. (1945). *Phytopathology*, **35**, 25–36.
Riehm (1940). *Plant Breed. Abstr.* **13**, 15–16.
Rieman, G. H. (1930). *Phytopathology*, **20**, 115–16.
Rieman, G. H. (1931). *J. Agric. Res.* **42**, 251–78.
Saboe, L. C. & Hayes, H. K. (1941). *J. Amer. Soc. Agron.* **33**, 463–70.
Salaman, R. N. & Lesley, J. W. (1923). *J. Genet.* **13**, 177–86.
Sansome, F. W. (1939). *Recent Advances in Plant Genetics*, 2nd ed. London: J. and A. Churchill.
Schreiber, F. (1932). *Plant Breed. Abstr.* **2**, 213.
Sengbusch, R. von (1938). *Plant Breed. Abstr.* **8**, 370.
Senn, P. H. (1932). *Phytopathology*, **22**, 675–97.
Shaw, F. J. F. (1936). *Indian J. Agric. Sci.* **6**, 139–87.
Shaw, F. J. F. & Bose, R. D. (1933). *Indian J. Agric. Sci.* **3**, 771–807.
Shen, T. H. (1934). *Bull. Coll. Agric. For. Nanking*, no. 17 (N.S.), pp. 1–16.
Shifriss, O., Myers, C. H. & Chupp, C. (1942). *Phytopathology*, **32**, 773–84.
Sieglinger, J. B. (1932). *J. Agric. Res.* **44**, 13–20.
Sieglinger, J. B., Swanson, A. F. & Martin, J. H. (1934). *J. Agric. Res.* **49**, 663–8.
Sinnott, E. W. & Hammond, D. (1930). *Amer. Nat.* **64**, 509–24.
Sirks, M. J. (1931). *Plant Breed. Abstr.* **2**, 218–20.
Spiegelmann, S., Lindegren, C. C. & Lindegren, A. (1945). Reported in *Nature, Lond.*, **157**, 448.
Stene, A. E. & Odland, T. E. (1938). *Proc. Amer. Soc. Hort. Sci.* (1937), **35**, 54–6.
Stephens, J. C. & Quinby, J. R. (1938). *J. Agric. Res.* **57**, 747–57.
Stevenson, T. M. & White, W. J. (1940). *Sci. Agric.* **21**, 18–28.
Straib, W. (1937). *Züchter*, **9**, 305–11.
'Student' (1933). *Eugen. Rev.* **24**, 293.
'Student' (1934). *Ann. Eugen.* **6**, 77.
Stummer, A. & Frimmel, F. (1932). *Plant Breed. Abstr.* **3**, 214.
Swenson, S. P. & Wells, D. G. (1944). *J. Amer. Soc. Agron.* **36**, 429–35.
Takahashi, N. (1934). *Plant Breed. Abstr.* **5**, 267.
Tavčar, A. (1935). *Plant Breed. Abstr.* **6**, 165.
Tavčar, A. (1939). *Plant Breed. Abstr.* **12**, 177.
Thadani, K. I. (1923). *Agric. J. India*, **18**, 572.
Ting, Y. (1933). *Agron. Bull. Coll. Agric. Sun Yatsen Univ. Publ.* Ser. III, **3**, 1–24.
Tingey, D. C. (1934). *J. Agric. Res.* **48**, 7, 631–55.

TORRES, J. P. (1937). *Philipp. J. Agric.* **8**, 27–40.
ULLSTRUP, A. J. (1941). *J. Agric. Res.* **63**, 331–4.
WADE, B. L. & ZAUMEYER, W. J. (1940). *J. Amer. Soc. Agron.* **32**, 127–34.
WADE, B. L., ZAUMEYER, W. J. & HARTER, L. L. (1938). *Circ. U.S. Dep. Agric.* no. 473, pp. 1–27.
WALKER, J. C. (1930). *J. Agric. Res.* **40**, 721–45.
WALKER, J. C., DELWICHE, E. J. & HARE, W. W. (1944). *Phytopathology* (Abstr.), **34**, 1013.
WATERHOUSE, W. L. (1930). *Proc. Linn. Soc., N.S.W.*, **55**, 595–636.
WATKINS, A. E. & CORY, F. M. (1931). *J. Genet.* **25**, 55–90.
WEETMAN, L. M. (1937). *Res. Bull. Ia Agric. Exp. Sta.* no. 228, 223–56.
WELLHAUSEN, E. J. (1937). *Res. Bull. Ia Agric. Exp. Sta.* no. 224, pp. 73–114.
WHITAKER, T. W. & PRYOR, D. E. (1941). *Phytopathology*, **31**, 534–40.
WINGARD, S. A. (1933). *Bull. Va. Agric. Exp. Sta.* **51**.
WOODWORTH, C. M. (1932). *Bull. Ill. Agric. Exp. Sta.* no. 384, pp. 297–404.
WORZELLA, W. W. (1934). *J. Agric. Res.* **49**, 705–14.
YEAGER, A. F. (1937). *J. Agric. Res.* **55**, 141–52.
YU, T. F. (1942). *Science Rec., Chungking*, **1**, nos. 1 and 2, pp. 248–50.

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